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Conference on *cis*-Platinum and Testicular Cancer

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2. **Correspondence regarding manuscripts previously published in *Cancer Treatment Reports*. Letters on other topics will be published at the discretion of the Editorial Board.**
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Begin the article with a short summary.

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ANNOUNCEMENT

Dr. Bruce A. Chabner assumed the post of Editor-in-Chief of *Cancer Treatment Reports* in July 1976, and the June issue of the Journal marked the completion of his 3 years of service. Dr. Chabner has gained the respect and admiration of the associate editors and the editorial staff for the perceptive and thoughtful way in which he guided the Journal through a period of unprecedented growth and change.

The obvious changes in format and design of *Cancer Treatment Reports* are familiar to all readers. However, during the last 3 years, far more important transitions in the Journal have occurred. From a journal with a relatively narrow emphasis on preclinical and clinical experimental chemotherapy, *Cancer Treatment Reports* has now matured under Dr. Chabner's leadership to a journal of general interest in the oncologic community. Contributions have ranged from studies on biochemical pharmacology and chemical carcinogenesis to studies on immunotherapy and sophisticated combined modality cancer treatment trials.

The Journal has grown in every way. The circulation for the calendar year 1978 has increased by 35% over the circulation for 1975. The number of articles submitted increased by 58% between 1975 and 1978. Major emphasis has been placed on the publication of proceedings of meetings dealing with such scientifically and clinically important subjects as anthracycline cardiotoxicity, treatment of non-Hodgkin's lymphomas, *cis*-platinum pharmacology and clinical results, and the role of immune modulation in oncologic research. Currently, at least four symposium issues are published each year.

Throughout the period of growth and change of *Cancer Treatment Reports*, Dr. Chabner has managed editorial matters with equanimity. He has suffered with patience and unfailing good humor the infrequent slings and arrows of outraged authors and associate editors. Those of us who worked under his leadership are appreciative of our association with him and we have gained from the experience. We thank him and wish him well.

Contents

Cancer Treatment Reports
Vol. 63, No. 9–10
September/October 1979

Proceedings of the National Cancer Institute Conference on *cis*-Platinum and Testicular Cancer

Page

Introduction—*cis*-Dichlorodiammineplatinum(II): The Trail From an Inert Electrode to a Therapeutic Solution 1431
Franco M. Muggia, Marcel Rozencweig, Vincent H. Bono, and Edwin M. Jacobs, Jr
National Cancer Institute, Bethesda, Maryland

Anticancer Activity of *cis*-Dichlorodiammineplatinum(II) and Some Relevant Chemistry 1433
Barnett Rosenberg
Michigan State University, East Lansing

Mechanism of Action of *cis*-Dichlorodiammineplatinum(II) 1439
Leonard A. Zwelling and Kurt W. Kohn
National Cancer Institute, Bethesda, Maryland

Mode of DNA Binding of *cis*-Platinum(II) Antitumor Drugs: A Base Sequence-Dependent Mechanism is Proposed 1445
Asher D. Kelman and Henry J. Peresie
Boston University School of Medicine, Massachusetts

Antitumor Activity of *cis*-Dichlorodiammineplatinum(II) 1453
Mary K. Wolpert-DeFilippes
National Cancer Institute, Bethesda, Maryland

***cis*-Dichlorodiammineplatinum(II): Combination Chemotherapy and Cross-Resistance Studies With Tumors of Mice** 1459
F. M. Schabel, Jr, M. W. Trader, W. R. Laster, Jr, T. H. Corbett, and D. P. Griswold, Jr
Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama

Platinate Toxicity: Past, Present, and Prospects 1475
A. M. Guarino, D. S. Miller, S. T. Arnold, J. B. Pritchard, R. D. Davis, M. A. Urbanek, T. J. Miller, and C. L. Litterst
National Cancer Institute, Bethesda, Maryland

Disposition and Distribution of Platinum Following Parenteral Administration of *cis*-Dichlorodiammineplatinum(II) to Animals 1485
Charles L. Litterst, André F. LeRoy, and Anthony M. Guarino
National Cancer Institute, Bethesda, Maryland

Page

Rationale for Development of Platinum Analogs 1493
Joseph H. Burchenal, Kathleen Kalaher, Kimberly Dew, and Linda Lokys
Memorial Sloan-Kettering Cancer Center, New York, New York

Structure-Activity Relationships of the Antitumor Platinum Coordination Complexes 1499
T. A. Connors, M. J. Cleare, and K. R. Harrap
Medical Research Council Laboratories, Carshalton, Surrey, England

Antileukemic (L1210) Activity and Toxicity of *cis*-Dichlorodiammineplatinum(II) Analogs 1503
A. W. Prestayko, W. T. Bradner, J. B. Huftalen, W. C. Rose, J. E. Schurig, M. J. Cleare, P. C. Hydes, and S. T. Crooke
Bristol Laboratories, Syracuse, New York

Platinum Analogs of Clinical Interest 1509
J. M. Hill, E. Loeb, A. Pardue, A. Khan, J. J. King, C. Aleman, and N. O. Hill
Granville C. Morton Cancer and Research Hospital, Wadley Institutes of Molecular Medicine, Dallas, Texas

Pharmacokinetics of Non-Protein-Bound Platinum Species Following Administration of *cis*-Dichlorodiammineplatinum(II) 1515
Robert J. Belt, Kenneth J. Himmelstein, Thomas F. Patton, Steve J. Bannister, Larry A. Sternson, and Arnold J. Repta
University of Kansas Medical Center, Kansas City

Nephrotoxicity of *cis*-Dichlorodiammineplatinum(II) 1523
Irwin H. Krakoff
Vermont Regional Cancer Center, University of Vermont, Burlington

Toxic Effects of *cis*-Dichlorodiammineplatinum(II) in Man 1527
Daniel D. Von Hoff, Richard Schilsky, Cheryl M. Reichert, Robert L. Reddick, Marcel Rozencweig, Robert C. Young, and Franco M. Muggia
National Cancer Institute, Bethesda, Maryland

***cis*-Dichlorodiammineplatinum(II)-Based Chemotherapy as Initial Treatment of Advanced Head and Neck Cancer** 1533
Robert Wittes, Keith Heller, Victor Randolph, Jane Howard, Alvaro Vallejo, Hollon Farr, Charles Harrold, Frank Gerold, Jatin Shah, Ronald Spiro, and Elliot Strong
Memorial Sloan-Kettering Cancer Center, New York, New York

***cis*-Dichlorodiammineplatinum(II) for the Treatment of Advanced Ovarian Cancer** 1539
Robert C. Young, Daniel D. Von Hoff, Paul Gormley, Robert Makuch, Jane Cassidy, Dianne Howser, and Joan M. Bull
National Cancer Institute, Bethesda, Maryland

Page

Cancer of the Ovary: A Summary of Experience With *cis*-Dichlorodiammineplatinum(II) at The Royal Marsden Hospital 1545
E. Wiltshaw, S. Subramarian, C. Alexopoulos, and G. H. Barker
The Royal Marsden Hospital, London, England

***cis*-Dichlorodiammineplatinum(II) in the Treatment of Gynecologic Malignancies: Phase II Trials by the Gynecologic Oncology Group** 1549
Tate Thigpen, Hugh Shingleton, Howard Homesley, Leo LaGasse, and John Blessing
Gynecologic Oncology Group

Phase II Evaluation of *cis*-Dichlorodiammineplatinum(II) in Advanced Malignancies of the Genitourinary and Gynecologic Organs: A Southwest Oncology Group Study 1557
Arthur H. Rossof, Robert W. Talley, Ronald Stephens, Tate Thigpen, Michael K. Samson, Carl Groppe, Jr, Harmon J. Eyre, and Richard Fisher
Southwest Oncology Group

Phase II Trials With *cis*-Dichlorodiammineplatinum(II) in the Treatment of Urothelial Cancer 1565
Alan Yagoda
Memorial Sloan-Kettering Cancer Center, New York, New York

Advanced Bladder Cancer: Therapy With *cis*-Dichlorodiammineplatinum(II), Adriamycin, and 5-Fluorouracil 1573
Stephen D. Williams, John P. Donohue, and Lawrence H. Einhorn
Indiana University Medical Center, and Veterans' Administration Hospital, Indianapolis

Phase II Trial of *cis*-Dichlorodiammineplatinum(II) in Cancer of the Bilharzial Bladder 1577
Nazli Gad-el-Mawla, Eric Chevlen, Reda Hamza, and John L. Ziegler
National Cancer Institute, Cairo, Egypt

Treatment of Genitourinary Tumors With *cis*-Dichlorodiammineplatinum(II): Experience in 250 Patients 1579
Claude E. Merrin
Roswell Park Memorial Institute, Buffalo, New York

***cis*-Dichlorodiammineplatinum(II) in Non-Small Cell Carcinoma of the Lung** 1585
Richard J. Gralla, Esteban Cvitkovic, and Robert B. Golbey
Memorial Sloan-Kettering Cancer Center, New York, New York

Phase II Study of Cyclophosphamide, Adriamycin, and *cis*-Dichlorodiammineplatinum(II) by Infusion in Patients With Adenocarcinoma and Large Cell Carcinoma of the Lung 1589
R. T. Eagan, S. Frytak, E. T. Creagan, J. N. Ingle, L. K. Kvols, and D. T. Coles
Mayo Clinic, Rochester, Minnesota

Page

cis-Dichlorodiammineplatinum(II) and VP-16-213: An Active Induction Regimen for Small Cell Carcinoma of the Lung 1593
John S. Sierocki, Basil S. Hilaris, Seymour Hopfan, Nael Martini, Deborah Barton, Robert B. Golbey, and Robert E. Wittes
Memorial Sloan-Kettering Cancer Center, New York, New York

Phase II Trial of cis-Dichlorodiammineplatinum(II) in Advanced Malignant Lymphoma and Small Cell Lung Cancer: Preliminary Results 1599
F. Cavalli, W. F. Jungi, R. W. Sonntag, N. I. Nissen, and J. F. Holland
Cancer and Acute Leukemia Group B

Phase II Evaluation of cis-Dichlorodiammineplatinum(II) in Lymphomas: A Southwest Oncology Group Study 1605
Arthur H. Rossof, Charles A. Coltman, Jr, Stephen E. Jones, and Robert W. Talley
Southwest Oncology Group

cis-Dichlorodiammineplatinum(II) in Adult Patients: Southwest Oncology Group Studies 1609
Ronald Stephens, Charles Coltman, Arthur Rossof, Michael Samson, Frank Panettiere, David Alberts, Muhyi Al-Sarraf, and John Bonnet
Southwest Oncology Group

Evaluation of cis-Dichlorodiammineplatinum(II) in Children With Advanced Malignant Diseases: Southwest Oncology Group Studies 1611
Teresa J. Vietti, Ruprecht Nitschke, Kenneth A. Starling, and Jan van Eys
Southwest Oncology Group

cis-Dichlorodiammineplatinum(II) in Childhood Cancer 1615
Arnold I. Freeman, Lawrence J. Ettinger, and Martin L. Brecher
Roswell Park Memorial Institute, Buffalo, New York

Phase II Study of cis-Dichlorodiammineplatinum(II) in Childhood Osteosarcoma: Children's Cancer Study Group Report 1621
Edward S. Baum, Paul Gaynon, Lowell Greenberg, William Krivit, and Denman Hammond
Children's Cancer Study Group

Germ Cell Tumors of the Testis 1629
B. L. Webber
National Cancer Institute, Bethesda, Maryland

Staging Procedures and the Role of Surgery in Testicular Cancer 1637
Nasser Javadpour
National Cancer Institute, Bethesda, Maryland

Applications of Biologic Tumor Markers in Testicular Cancer 1643
Nasser Javadpour
National Cancer Institute, Bethesda, Maryland

Page

Radiotherapy in the Management of Nonseminomatous Testicular Carcinoma 1649
Eli Glatstein
National Cancer Institute, Bethesda, Maryland

An Appraisal of the Role of Radiation Therapy in the Management of Nonseminomatous Germ-Cell Tumors of the Testis in the Era of Effective Chemotherapy 1653
M. J. Peckham
The Institute of Cancer Research and The Royal Marsden Hospital, Sutton, Surrey, England

Combination Chemotherapy With *cis*-Dichlorodiammineplatinum(II) in Disseminated Testicular Cancer 1659
Lawrence H. Einhorn
Indiana University Medical Center, and Veterans' Administration Hospital, Indianapolis

Vinblastine, Bleomycin, and *cis*-Dichlorodiammineplatinum(II) in Disseminated Testicular Cancer: Preliminary Report of a Southwest Oncology Group Study 1663
Michael K. Samson, Ronald L. Stephens, Saul Rivkin, Michael Opipari, Thomas Maloney, Carl W. Groppe, and Richard Fisher
Southwest Oncology Group

Treatment of Metastatic Nonseminomatous Testicular Cancer: A Preliminary Report of Induction Chemotherapy Followed by Maintenance Chemotherapy or Radiotherapy 1669
Roland W. Sonntag, Hans Jörg Senn, and Franco Cavalli
University Clinics, Inselspital, Bern, Switzerland

Chemotherapy for Advanced Germinal Cell Neoplasms: Preliminary Report of an Eastern Cooperative Oncology Group Study 1675
William D. DeWys, Colin Begg, Robert Slayton, Richard G. Hahn, and Isadore Brodsky
Eastern Cooperative Oncology Group

Sequential Combination Chemotherapy and Surgery for Disseminated Testicular Cancer: *cis*-Dichlorodiammineplatinum(II), Vinblastine, and Bleomycin Remission-Induction Therapy Followed by Cyclophosphamide and Adriamycin 1681
Marc B. Garnick, George P. Canellos, Jerome P. Richie, and James J. Stark
Sidney Farber Cancer Institute, Boston, Massachusetts

Chemotherapy for Testicular Cancer: Current Status of the National Cancer Institute Combined Modality Trial 1687
Tom Anderson, Nasser Javadpour, Richard Schilsky, Audrey Barlock, and Robert C. Young
National Cancer Institute, Bethesda, Maryland

Page

Basis for Adjuvant Chemotherapy for Stage II Testicular Cancer 1693
William D. DeWys
Northwestern University School of Medicine, Chicago, Illinois

ANNOUNCEMENTS 1697

EORTC Conference on Metastasis: Clinical and Experimental
2nd Workshop on Cloning Human Tumor Stem Cells
International Symposium on New Trends in Human Immunology and Cancer Immunotherapy
Postgraduate Course on Metabolic and Nutritional Care
1978 Manual for Staging of Cancer
American Board of Internal Medicine Examinations
European Symposium on Lung Cancer
Symposium on the Nutrition of the Cancer Patient

The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1980.

Proceedings of the National Cancer Institute Conference on *cis*-Platinum and Testicular Cancer

Coordinator: Marcel Rozencweig

Symposium Editors: Tom Anderson
Nasser Javadpour
Leonard Zwelling

September 21–22, 1978
Washington, DC

Introduction

cis-Dichlorodiammineplatinum(II): The Trail From an Inert Electrode to a Therapeutic Solution

Franco M. Muggia, Marcel Rozencweig, Vincent H. Bono, and Edwin M. Jacobs, Jr [1]

This issue will comprehensively cover the development and clinical evaluation of a new anticancer agent, the heavy metal derivative, *cis*-dichlorodiammineplatinum(II) (*cis*-platinum). After thorough laboratory studies, the National Cancer Institute introduced *cis*-platinum into clinical investigation in 1971. Initial experience identified some antitumor activity in man, as well as dose-limiting renal toxicity. Methods to circumvent nephrotoxicity by close monitoring of hydration and urinary excretion rates eventually allowed the use of safer and higher dose regimens. These methods have resulted in establishing the efficacy of *cis*-platinum in the treatment of various tumor types, particularly testicular cancer. Results achieved with *cis*-platinum in this most aggressive malignancy affecting young men have far-reaching implications regarding curability. Treatment of this cancer, therefore, formed the basis of a separate section of the conference, and is covered by several papers ranging from pathology, biologic markers, and staging to surgery, irradiation, and chemotherapy.

Numerous areas of investigation related to *cis*-platinum are currently under intensive scrutiny. Among these are questions regarding knowledge of its mechanism of action, the development of related compounds, the further delineation of its spectrum of activity, and the presence of synergistic interaction with other treatment modalities.

This overview should provide oncologists with an appreciation of current and future directions which have emanated from the introduction of a new effective drug in cancer chemotherapy.

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Anticancer Activity of *cis*-Dichlorodiammineplatinum(II) and Some Relevant Chemistry

Barnett Rosenberg [1,*]

Anticancer Activity of Platinum Coordination Complexes

Metal coordination chemistry, particularly that of the platinum group metals, is a fertile field in the search for new anticancer drugs. In a random screen of compounds, recent evidence suggests that we are 25 times more likely to find active drugs among metal complexes than among organic compounds. One of the first such complexes to be seriously investigated, *cis*-dichlorodiammineplatinum(II) (*cis*-platin), has proved to be a broad-spectrum, potent anticancer agent in animals, and to be of significant value in the treatment of some human cancers.

The discovery of the biologic effects of *cis*-platin constitutes a classic case of serendipity in research. The history of this discovery has already been told (1). The compound, known as Peyrone's chloride, was first synthesized in 1845. Its biologic activity was first determined (2) in 1967 as a curious effect on bacteria; it stopped cell division but not growth, thus leading to giant filamentous cells. With this biologic assay, it was easy to probe the nature of the chemicals of this class. The early results show a diversity of bacterial actions depending on the structures of the complexes tested. Charged species of platinum complexes tended to be bacteriocidal. Neutral species were the filament formers, which also induced lysis (both directly and indirectly [3]) in lysogenic strains of bacteria. It is now known, of course, that it is the neutral species of platinum complexes, almost without exception, that exhibit anticancer activity, and that some correlation exists between both the filamentation and lytic effects in bacteria and the anticancer activity in animals. The structures of some of the earliest drugs found are shown in figure 1. These exist in both of the usual valence states of platinum: II, forming square planar complexes, and IV, forming octahedral complexes. The active leaving groups (chlorides, in most instances) can be arranged on the same side of the molecule (*cis* configurations) or diametrically opposite each other (*trans* configurations).

We knew at an early stage that only the *cis* configurations showed the interesting biologic actions; the *trans* configurations never did, and so far, never have. Thus, these structure-activity studies have led to a set of "rules of thumb" for the anticancer activity of platinum complexes. These rules are: (*a*) the complexes exchange only some of their ligands in reactions with biologic molecules; (*b*) the molecules should be neutral; (*c*) the exchangeable (leaving) groups should be in the *cis* configuration; (*d*) the tightly bound ligands should preferentially be relatively inert amines; and (*e*) the lability of the leaving groups should be neither too high nor too low, but roughly centered on that of chlorides.

Both the correlations with the bacterial effects and the *cis* versus *trans* stereochemistry are sufficiently well-documented to provide secure bases for speculations on the mechanism of action of these drugs as anticancer agents. The lytic effects of drugs are not yet understood in molecular detail, but they quite obviously involve at some stages (*a*) the derepression of latent viral genomic information (4,5), and (*b*) the enhanced production of virally coded proteins and their consequent appearance at the cell surface leading to a possible increase in the antigenicity of the cell (6). If one could be sure that latent viruses are relevant to human cancers, this would be an interesting hypothesis. In any case, the correlations do add significant evidence to that obtained more directly (7,8): that interaction of the platinum molecules with DNA is the primary effect on the cell leading to anticancer activity. It must be accepted, however, that simply reacting with DNA is not sufficient to account for the specificity of anticancer drugs. After all, large tumors are made to disappear in patients, without unacceptable toxic effects to the normal cells. While one may cite many arguments as to why this may be, they are all in the nature of "hand waving." We must eventually address ourselves directly to this point.

The stereospecificity of this *cis* versus *trans* configurational necessity is at the molecular level and, therefore, provides a more logical starting place to look for molecular mechanisms of action. Here too, however, there is a fundamental limitation; no matter how many chemical

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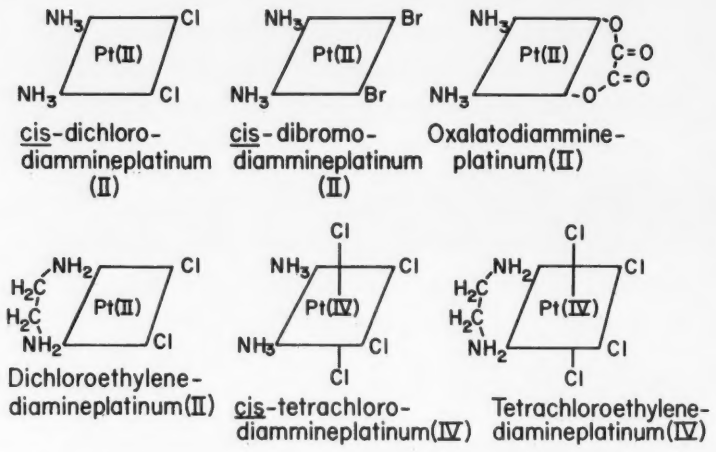


FIGURE 1.—Molecular structures of the 1st group of active anticancer platinum coordination complexes.

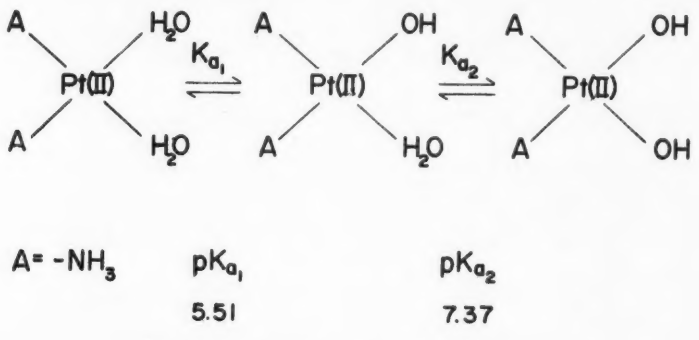


FIGURE 2.—Aquated species of *cis*-platin.

studies we do, medical results cannot be explained without addressing biologic processes. A lesion on a cancer cell's DNA is no different than a lesion on a normal cell's DNA. Somehow the malignant and the normal cells must process these lesions differently.

Aquation Reactions of *cis*-Platin

When *cis*-platin is dissolved in water, the two chloride leaving groups sequentially exchange for water or hydroxy ligands, depending on the pH. At a temperature of 37°C, the half-life for the completion of this reaction is 1.7 hours. The activation enthalpy is approximately 20 kcal/mol. This reaction is shown in equation 1:

$$cis\text{-}Pt(II)(NH_3)_2Cl_2 \xrightarrow{H_2O} cis\text{-}Pt(II)(NH_3)_2Cl(H_2O)^+ \longrightarrow cis\text{-}Pt(II)(NH_3)_2(H_2O)_2^{2+} . \quad [1]$$

When a high amount of chloride is present, eg, isotonic saline (0.9%), the aquation reaction does not occur to any significant extent, and the drug remains in the neutral form. Thus, in extracellular fluid, the drug is unchanged, and in mice, it is excreted rapidly via the urine (half-life in man of 1.5 hours) with 95% in the neutral *cis*-platin form. For some cells, the intracellular fluid has a chloride concentration about one thirtieth of the extracellular fluid (eg, in muscle cells). At these concentrations the aquation reaction proceeds, and within a few hours, the drug is present as the monochloro-monoaquo or diaquo form. These are the chemicals that must react with the cellular receivers, presumably DNA. Depending upon the pH, the diaquo form may exist as three different structures. These are shown in figure 2. At approximately neutral pH, the species most likely to be formed is the monoaquo-monohydroxy. Thus, inside the cell the monochloro-

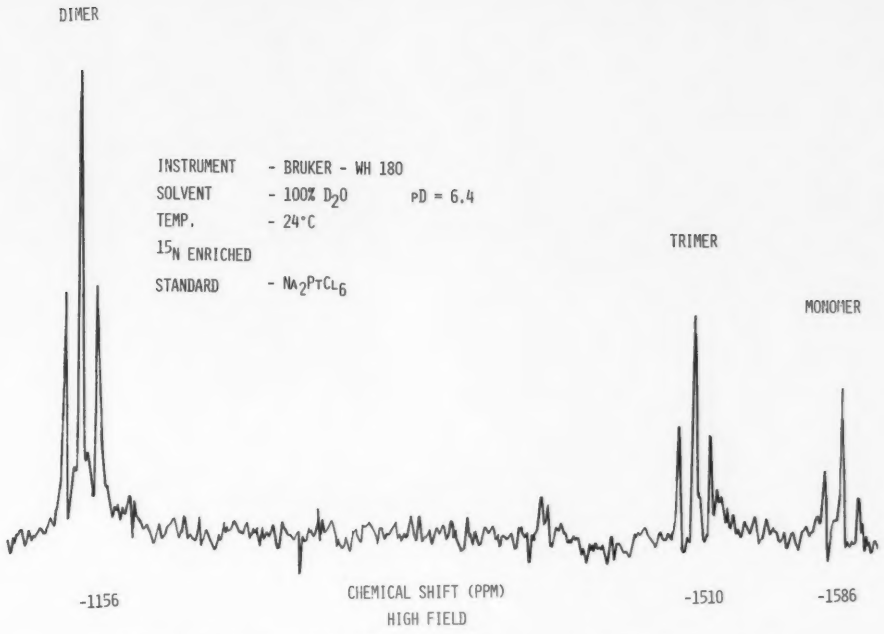


FIGURE 3.—^{195}Pt(II)-FT-NMR spectra of the monomer, dimer, and trimer of [*cis*-Pt(II)$(NH_3)_2(OH)(H_2O)]\cdot NO_3$.

monoaquo, the monochloro-monohydroxy, and the monoaquo-monohydroxy forms will appear. This complicates the attempt to understand the further chemical reactions.

Recently, Lippert et al (9) and Faggiani et al (10–12) have shown that the aquated species of *cis*-platin oligomerizes readily. A monomer, a hydroxo-bridged centrosymmetric dimer, and a hydroxo-bridged trimer can all be crystallized, and their structures have been determined by X-ray crystallography. The monomer is stable at very low (as the diaquo form) and very high (as the dihydroxy form) pHs. Near neutral pH, the monomer oligomerizes readily. Chikuma and co-workers[2] have measured the kinetics and the thermodynamics of these reactions by the use of ^{195}platinum-nuclear magnetic resonance (NMR). Figure 3 shows the nature of the NMR signals from each of these species after some time of reaction. Figure 4 shows the kinetics of the oligomerization reactions at a pH near neutrality. Finally, figure 5 shows the half-life for disappearance of the monomer (at a solution concentration of 0.05 M) as a function of pH. It is obvious that the fastest reaction occurs with the *cis*-platin monomer in the monoaquo-monohydroxy form. This is precisely the form we expect to find inside cells. This raises very serious questions concerning the possibility of a new set of reactions which may compete with monomer-cell receiver interactions and concerning the

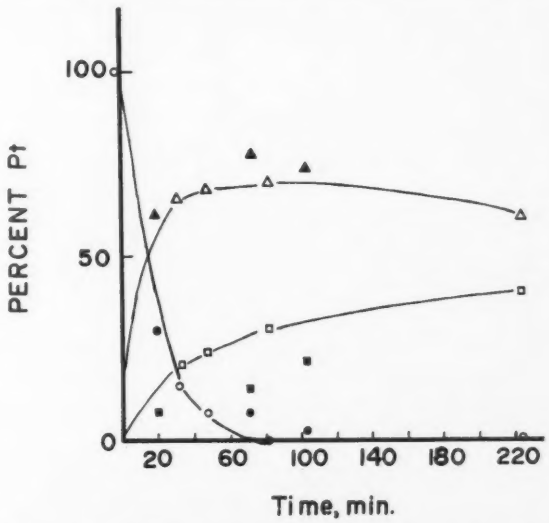


FIGURE 4.—^{195}Pt(II)-FT-NMR-determined kinetics of the oligomerization reaction of the monomer, *cis*-Pt(II)$[(NH_3)_2(OH)(H_2O)]^+$ in PIPES buffer at pH 6.60 and in water. Monomer = ○,●; dimer = △,▲; trimer = □,■ (solid symbols are without buffer).

[2]Chikuma M, Pollock RJ, Ott KC, et al. Hydroxo-bridged platinum(II) complexes. Kinetic studies on olation reaction of *cis*-diaquodiammineplatinum(II) by ^{195}Pt Fourier transform nuclear magnetic resonance. Manuscript submitted for publication to J Am Chem Soc, 1979.

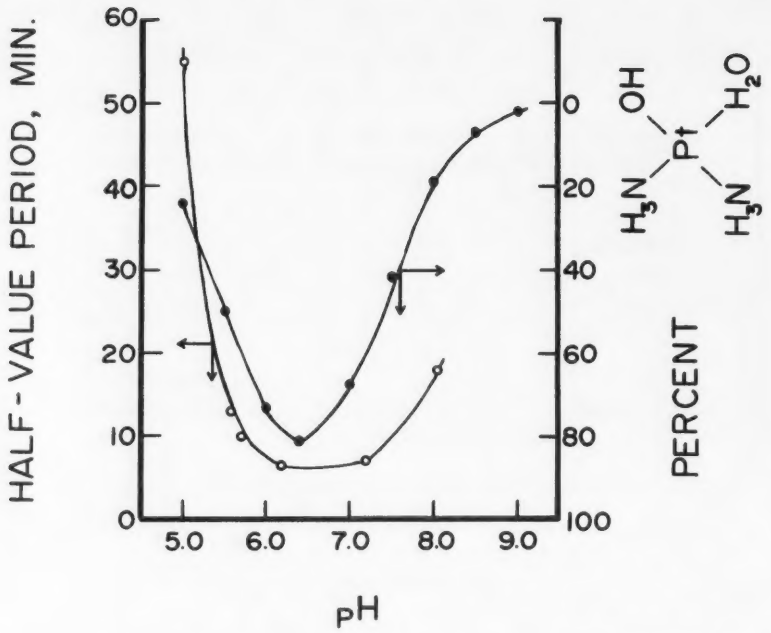


FIGURE 5.—Half-life (mins) for the disappearance of the monomer, *cis*-Pt(II)[$(NH_3)_2(OH)(H_2O)]^+$, ^{195}Pt(II)-FT-NMR signal, and % concentration of this form of the aquated species as functions of the pH.

relative anticancer activity or cytotoxicity of the dimeric and trimeric species. These latter have been studied, by injecting each oligomer directly (ip) into animals. This is perhaps not the best way to study these species, since the high chloride concentration of extracellular fluids will affect the various forms differently. It is sufficient, however, to give us a clue to their diverse actions.

Briefly, the preliminary results suggest that all three are more toxic (lower LD50) than *cis*-platin, with the monomer the least toxic (producing deaths only after 3 days [as do excessive doses of *cis*-platin]), while high doses of the dimer and trimer are immediately lethal. These latter oligomers do not display anticancer activity at any dose levels, while the monomer is very active at dose levels below the frankly toxic level. These results may be of some relevance to the cytotoxicity to those normal cells which have large concentrations of *cis*-platin accumulated, such as the kidney, in which the dimerization rate (which increases as the square of the monomer concentration) may be significant. These reactions may also provide a molecular rationale for the lower toxic effects (but maintained efficacies) of *cis*-platin given as a divided dose (over 24 hours) (13) or as a slow infusion over 24 hours.

Speculations on the Mechanism of Action of *cis*-Platin

I have earlier alluded to the stereospecific requirements of a *cis* configuration for anticancer activity of the platinum coordination complexes. The best quantitative data to support this have come from the cooperative studies of Braddock et al (14). Their animal results, using the ADJ/PC6A myeloma in C^- mice, are shown in table 1. For a variety of amine ligand substitutions on the dichloroplatinum(II) basic structure, the activities (therapeutic indices) vary over a range of > 30–1. The *trans* configurations are not active until one reaches the low toxicity cyclopentylamine analog, and here, the small activity shown may well be due to an almost unavoidable small amount of the *cis* analog contaminating the *trans* configuration preparation.

If we make the reasonable assumption that the chemistry and transport of the *cis* isomers will not be too dissimilar from those of the *trans* isomers, we must look to the cell receivers for the necessary site restriction that uniquely picks the *cis* isomers to show anticancer activity. The preponderance of evidence, reviewed recently by Roberts and Thomson (15), leads to the hypothesis that

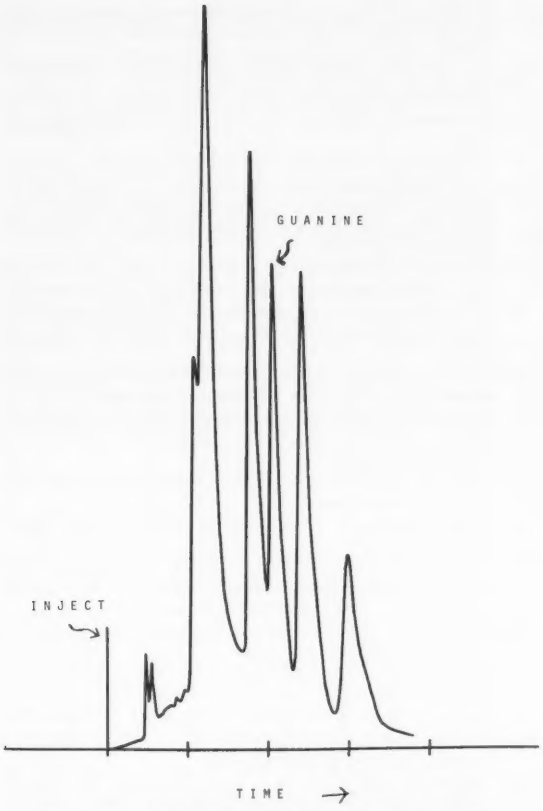
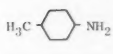


FIGURE 6.—High performance liquid chromatographic spectrum of the reaction products of aquated, monomeric *cis*-platin with guanine.

TABLE 1.—Anticancer activity of *cis* and *trans* isomers of $Pt(am)_2Cl_2$ complexes using the tumor system ADJ/PC6A in C^- mice (*from* ref 14)

am	Isomer	LD50 (mg/kg)	ID90 (mg/kg)	Therapeutic index (LD50/ID90)
NH_3	*cis*	13	1.6	8.1
	trans	27	> 27	—
	cis	56.5	2.6	21.7
	trans	18.0	> 18.0	—
	cis	240	17.5	13.7
	trans	72	> 72	—
	cis	57	2.3	25
	trans	27	> 27	—
	cis	90	2.9	31
	trans	250	> 110	—
	cis	480	2.4	200
	trans	180	72	2.5
	cis	> 3200	12	> 267
	trans	680	260	2.6
	*cis*	990	1180	—
	trans	360	> 800	—

the interaction of *cis*-platin with DNA is the essential cell lesion. This becomes a difficult problem to study when the multiplicity of possible reactions between these molecules is considered. Nevertheless, many laboratories have been, and are now, studying these reactions. About the only reaction that can be excluded is that of intercalation. This comes both from direct in vitro studies and from the lack of mutagenic action of *cis*-platin against the Ames bacterial tester strains which are specific for frame shift mutations. All other conceivable reactions have been sought, and most have been found. Again, where *cis* versus *trans* actions have been included in the studies, little difference has been found. Both interstrand (DNA-DNA and DNA-protein) and intrastrand cross-links have been detected. Reactions with phosphate groups (although of uncertain relevance) but not with sugar groups of DNA have been determined. Reactions with individual bases, with G-C pairs as the most likely sites, occur.

However, the most exciting suggestion, independently put forward by Macquet and Theophanides (16), Goodgame et al (17), and Dehand and Jordanov (18), is that of an N7–O6 chelate complex on guanine. This could exhibit the requisite stereospecificity, and, moreover, has the added value of involving the O6 site of guanine which has been shown to be the most likely site of alkylation leading to carcinogenesis. It can do this by remaining unrepaired in some cells deficient in the necessary repair enzyme (19) until DNA replication has occurred, with a consequent mispairing of guanine with thymidine. Upon a second replication at least one of every four such sites will lead to a T-A pair where previously a G-C pair existed. This is a base substitution mutation. We had predicted that this course of events should lead to the *cis* isomer being a base substitution mutagen, while the *trans* would not be. This has now been confirmed by Beck and Fisch[3] using the Ames tester strains, and by Zwelling et al (20) using mammalian cells. Unfortunately, confirmation of the prediction cannot be considered verification of the hypothesis, since other hypotheses may conceivably lead to the same prediction. These results are, however, at least consistent with the N7–O6 chelate complex.

Chemical evidence for the existence of this chelate complex is not considered sufficiently strong. All nucleophilic sites of guanine are available for attack by the electro-

[3]Beck DJ, and Fisch JE. Mutagenicity of platinum coordination complexes in *Salmonella typhimurium*. *Mutat Res*, 1979. In press.

philic platinum complex, and all such reaction products will probably be found. We should take advantage of what has been patiently learned from work with alkylating agents, that while N7 may be the predominant attack site chemically, it is not the relevant lesion for carcinogenesis. The biologic processing chooses a minor component, the O6 lesions. We have begun a study of this in my laboratory, using the high performance liquid chromatographic separation of the reaction products of *cis*-platin with guanine. Figure 6 shows a chromatogram of a preliminary result. The large number of peaks and shoulders indicate that, indeed, a multiplicity of products has formed. It will take some time to sort these out and determine the structures of each product; their relevance to the anticancer activity of *cis*-platin will take a little longer.

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Mechanism of Action of *cis*-Dichlorodiammineplatinum(II)

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SUMMARY

Although the mechanism of action of *cis*-dichlorodiammineplatinum(II) is not definitely known, much has been discovered regarding its antitumor effect. Although neutral in plasma, the platinum complex becomes aquated intracellularly to form a positively charged platinum species that is likely to interact with nucleophilic sites. The purine and pyrimidine bases of DNA possess such sites and provide the locale for the majority of the biologically effective intracellular platinum binding. Although several types of binding are possible, the biologically important lesions are most likely interstrand and/or intrastrand cross-links. The extent of formation and the rate of cellular repair of these lesions may determine the ultimate effect of *cis*-dichlorodiammineplatinum(II) on the cell.

[Cancer Treat Rep 63:1439–1444, 1979]

The study of the chemistry of square planar coordination complexes of platinum began with the discovery of two platinum-chloride salts by Reiset in 1844 and Peyrone in 1845 (1). In 1893, Werner suggested that these two compounds were geometric isomers and this has since been confirmed. Peyrone's salt is now known as *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) and Reiset's salt is its *trans* isomer (fig 1).

It was not until the publication of the landmark papers by Rosenberg et al in 1965 and again in 1969 that the ability of platinum compounds to inhibit cell and tumor growth was recognized (2,3). As has occurred with many other pharmacologic agents, the elucidation of the site and mechanism of the molecular action of *cis*-platinum lagged behind the observation of its biologic effects upon cells and whole organisms and even trailed its introduction into clinical oncology. This paper summarizes some of the current ideas relating to the molecular mechanism of the tumoricidal action of *cis*-platinum.

Geometry and Chemistry

The antineoplastic activity of *cis*-platinum appears to be related to the chemical nature of the species which are bound to the central platinum atom and the relative position of these ligands to one another. Active platinum compounds must have two sites at which they can interact with intracellular targets, and these two sites must be in the *cis* configuration, that is, adjacent to one another, rather than the *trans* configuration, or opposite one another (4,5) (fig 1). Because reactions with molecules within the cell rather than at the cell surface are important to the cytotoxicity of *cis*-platinum, the drug must be able to penetrate the cell membrane prior to exerting its intracellular tumoricidal action.

The adjacent chloride ligands are labile sites in *cis*-platinum which can be replaced in a fashion analogous to that described for the leaving groups of bifunctional alkylating agents (6). However, in the relatively high chloride ion content of circulating blood (103 mM), *cis*-platinum remains electrically neutral (4) (fig 2). Preserving this neutrality is important since moieties that are electrically charged in the milieu of the plasma probably would not traverse membranes without the aid of carrier-mediated transport, and no such transport has been described for *cis*-platinum (7,8). Inside the cell the chloride ion concentration is lower (4 mM), and the chloride leaving groups are replaced by water molecules resulting in a positively charged, aquated platinum species (fig 2) which can react with nucleophilic sites within the cell in a manner analogous to that of bifunctional alkylating agents (4,6).

The NH_3 ligands are strongly bound to the central metal atom and are unlikely to participate in *cis*-platinum's reactions with biomacromolecules (4). However, Burchenal et al (9) have reported that platinum com-

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[2]We gratefully thank Madie Tyler for the preparation of the manuscript.

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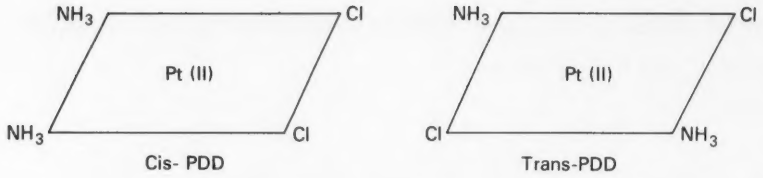


FIGURE 1.—Structures of *cis*-platinum and *trans*-platinum.

pounds which have had their NH_3 ligands replaced by a 1,2-diamminocyclohexane moiety are active against animal tumors resistant to *cis*-platinum. Thus, the role of the NH_3 ligands in the antitumor action of *cis*-platinum may also be important, but awaits further delineation.

DNA as the Target of *cis*-Platinum

There are now several lines of evidence that point to DNA as the intracellular target for *cis*-platinum (fig 3). In bacteria, *cis*-platinum inhibited cell division (2), reduced viability of DNA repair-deficient mutants to a greater extent than wild-type organisms (10), induced prophage from lysogenic *Escherichia coli* (11), and was itself mutagenic (12). *cis*-Platinum inactivated transforming DNA (13) and DNA-containing bacteriophage (14). In mammalian cells, *cis*-platinum selectively inhibited DNA synthesis in Ehrlich ascites tumor cells (15), human amnion AV_3 cells (16), and human leukocytes (17). New evidence that *cis*-platinum acts upon DNA is that *cis*-platinum was mutagenic in mammalian cells (18), and that xeroderma pigmentosum fibroblasts, deficient in DNA repair, were more susceptible to the toxic effects of *cis*-platinum than were normal fetal lung cells (19).

On the molecular level, Pascoe and Roberts (20) have shown that more platinum molecules were bound per molecule of DNA than per molecule of RNA or protein in HeLa cells treated with *cis*-platinum. More recently, Roberts and Fraval[3] reported that the extent of platinum binding was comparable for the DNA of cells treated in vitro with toxic doses of *cis*-platinum and for the DNA of tumor cells treated in vivo with doses causing tumor regression. Lastly, Harder et al (21) demonstrated that the depression of DNA synthesis produced by *cis*-platinum was not due to inhibition of DNA polymerase, but rather to drug action on the DNA template (21).

Since Pascoe and Roberts (20) demonstrated that at equitoxic doses of *cis*- and *trans*-platinum, more of the less active *trans* isomer was bound to DNA than the *cis* isomer, and assuming that the lethality of these compounds derives from DNA binding, *cis*-platinum produces a more lethal class of lesion either by binding to sites to which *trans*-platinum does not bind, by producing lesions structurally different from those produced by

[3]Roberts JJ, and Fraval HNA. The relationship between the levels of interaction of antitumor compounds with cellular DNA and their cytotoxic activities. Presented at the EUCHEM Conference on Coordination Chemistry and Cancer Chemotherapy, Toulouse, France, July 24–26, 1978.

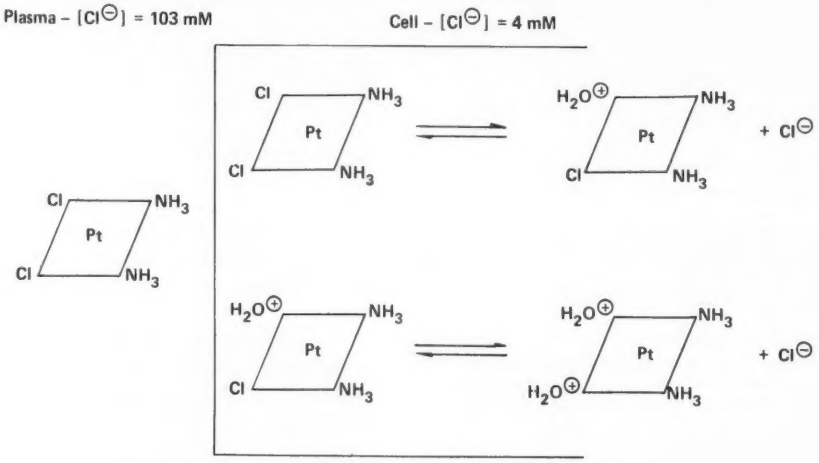


FIGURE 2.—Aquation of *cis*-platinum.

1. Inhibited bacterial cell division
2. Reduced viability of bacteria deficient in DNA repair as compared to wild-type organisms
3. Induced prophage from lysogenic bacteria
4. Induced mutations in bacteria
5. Inactivated transforming DNA
6. Inactivated DNA-containing bacteriophage
7. Selectively inhibited DNA synthesis in several mammalian cell systems including human cells
8. Induced mutations in mammalian cells
9. Reduced viability of human cells deficient in DNA repair (xeroderma pigmentosum cells) compared to normal fetal lung cells
10. Preferentially bound to intracellular DNA
11. Toxic doses of cis-platinum in vitro and tumoricidal doses of cis-platinum in vivo produced comparable DNA-platinum binding
12. Inactivated DNA template for DNA polymerase

FIGURE 3.—Evidence supporting DNA as the major intracellular target for *cis*-platinum.

trans-platinum, or by producing lesions which the cell cannot remove.

Types of DNA Interactions With *cis*-Platinum

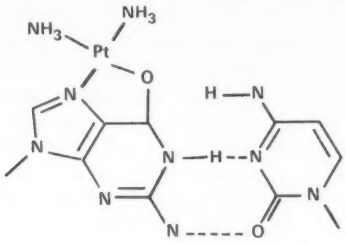
It has been generally accepted that it is the reactions with the purine and/or pyrimidine bases rather than the sugar-phosphate moieties of DNA that are important to the antitumor action of *cis*-platinum (22–24). The aquated platinum species would probably react with nucleophilic sites within DNA bases in a fashion analogous to that of classic alkylating agents. The sites within the various bases to which *cis*-platinum binds and their relative importance to the antineoplastic action of the compound have not been conclusively discerned. A recent review (25) summarized the important data.

cis-Platinum can bind to DNA bases in several possible ways (fig 4). It can bind to a single nucleophilic site (monofunctional binding). Bifunctionally, it can bind to two sites within a single DNA base or between two bases. Two bases bound or cross-linked by a single platinum-containing molecule can be on one DNA strand (intra-strand cross-link) or on opposite DNA strands (inter-strand cross-link). Cross-links between DNA and other biomacromolecules, such as proteins, could also be formed.

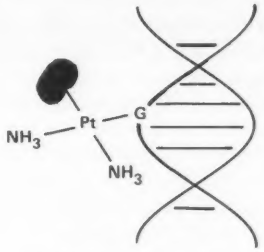
Monofunctional binding is unlikely to be the manner in which *cis*-platinum exerts its tumoricidal action, since Pascoe and Roberts (20) have shown that at equitoxic doses more *trans*-platinum than *cis*-platinum is bound to DNA.

Evidence for bifunctional binding to a single base has been reported for *cis*-platinum but not for *trans*-platinum (26,27). Such a reaction could interfere with base pairing and could account for the inhibition of DNA function characteristic of *cis*-platinum.

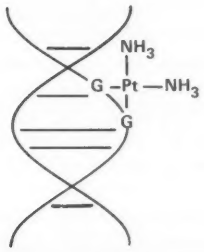
Intrastrand cross-linking has also been proposed as an



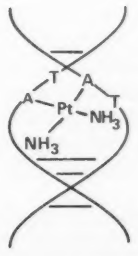
Bifunctional Binding to 1 DNA Base



DNA-Protein Crosslink



Intrastrand Crosslink



Interstrand Crosslink

FIGURE 4.—Possible types of DNA interactions with *cis*-platinum.

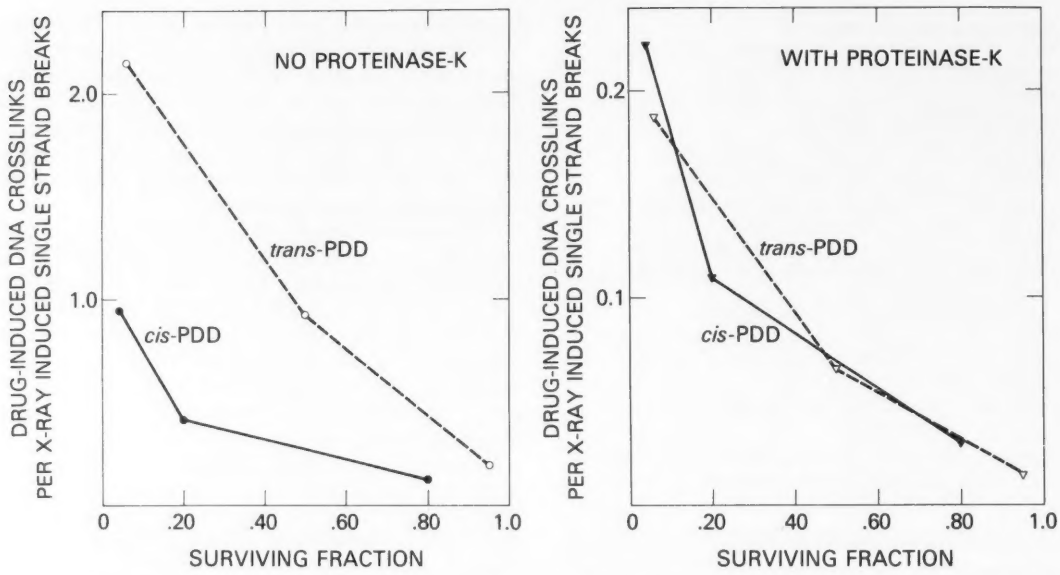


FIGURE 5.—DNA cross-linking vs cytotoxicity in L1210 cells treated with *cis*-platinum or *trans*-platinum. Total cross-linking is on left; proteinase-resistant cross-linking is on right (*see* text).

important lesion produced by platinum compounds. The evidence for its occurrence derives from in vitro experiments in which the binding of intercalating compounds to DNA can be decreased by prior reaction of the DNA with active platinum compounds (28,29). This was felt to imply that the drug maintained the base stacking through some type of cross-linking, presumably intrastrand or interstrand. Because interstrand cross-linking was thought to be a rare event (13,14), the intrastrand cross-link was felt to be excluding the intercalator.

More recent evidence for the importance of intrastrand cross-linking to *cis*-platinum action is that *cis*-platinum, but not *trans*-platinum, preferentially increased the buoyant density of poly(dG)·poly(dC), but not that of poly[d(G-C)], suggesting site specificity for G-G pairs (30). The possible site specificity of *cis*-platinum action is further suggested by results recently reported by Kelman et al.[4] They utilized the DNA restriction endonuclease Bam-H1 which specifically cleaves the deoxypentose phosphate bond between adjacent guanine bases in the restriction sequence GGATCC, and examined the gel patterns of the resultant cleavage fragments from lambda DNA before and after treatment of the DNA with ethylenediammineplatinum(II) (EDP), an active analog of *cis*-platinum. The gel pattern generated by Bam-H1 digestion of untreated lambda DNA was prevented by pretreating the DNA with EDP. However, the pattern was restored if the Bam-H1 digest of the EDP-treated DNA was dialyzed against CN^- which was thought to remove DNA-bound platinum. Kelman et al speculated that although the Bam-H1 could cleave the bond between adjacent guanines in EDP-treated DNA, the platinum bridged the bases and prevented their separation until it was removed. Thus, DNA intrastrand cross-linking, most probably at adjacent guanine pairs, may prove to be an important cytotoxic lesion produced by *cis*-platinum.

As stated above, the interstrand cross-link has been dismissed by several investigators (13,14) as being too uncommon an event to account for the biologic activity of *cis*-platinum. However, Pascoe and Roberts (20) pointed out that interstrand cross-linking might prove to be important since experiments reporting cross-linking as a rare event had examined DNA only immediately after treatment and that cross-link formation could increase with time. Zwelling et al (31) have recently reported precisely this phenomenon in L1210 cells treated with *cis*-platinum and examined by DNA alkaline elution. The maximum cross-linking produced by *cis*-platinum appeared 12 hours after a 1-hour treatment.

By modifying this technique to include proteinase digestion as well as cell lysis prior to DNA alkaline elution, two classes of cross-linking have been discerned (18). That cross-linking which is sensitive to proteinase digestion was taken to represent DNA-protein cross-linking. That cross-linking which remains after proteinase digestion was felt to include interstrand cross-linking.

[4]Kelman AD, Peresie HJ, Buchbinder M, et al. Molecular mechanisms of platinum and ruthenium antitumor complexes. Presented at the EUCHEM Conference on Coordination Chemistry and Cancer Chemotherapy, Toulouse, France, July 24–26, 1978.

Comparing the two classes of cross-linking produced by the active *cis*-platinum and the less active *trans*-platinum with their respective abilities to inhibit colony formation by L1210 cells revealed no correlation between total cross-linking and cytotoxicity (fig 5). Since most of the total cross-linking was sensitive to proteolytic digestion, the DNA-protein cross-link was not felt to be an important cytotoxic lesion produced by these compounds. There was, however, good correlation between proteinase-resistant cross-linking and cytotoxicity (fig 5). Thus, the interstrand cross-link cannot be excluded as a candidate for the cytotoxic lesion produced by platinum compounds.

Possible Role of DNA Repair

Finally, we still must account for the preferential toxicity of *cis*-platinum to tumor cells when compared to normal cells, for it is this therapeutic index which allows its clinical use. A common explanation for differential toxicity is that tumor cells are more rapidly proliferating than normal cells and are more rapidly synthesizing DNA. Drug inhibition of DNA synthesis would thus be more toxic to rapidly dividing tumor cells. Roberts and Fraval[5] have recently demonstrated that stationary phase cultures were more sensitive than exponentially growing cultures. Stationary phase cells were also far inferior to exponentially growing cells in the excision of platinum from their DNA.[6] Xeroderma pigmentosum cells, deficient in DNA repair, were more sensitive than normal cells (19). Thus, the ultimate consequence of *cis*-platinum's action upon any cell might result not only from the site and manner of intracellular binding but also from the potential of that cell to repair the drug-inflicted damage. Repair of *cis*-platinum damage has been inferred from the results of several investigations (18,31–33) and may well hold the key to the development of newer, more active analogs of *cis*-platinum.

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Mode of DNA Binding of *cis*-Platinum(II) Antitumor Drugs: A Base Sequence-Dependent Mechanism is Proposed [1]

Asher D. Kelman* and Henry J. Peresie [2,3]

SUMMARY

Chloroammine and similar complexes of platinum(II) having the ammine ligands in the *cis* configuration are effective antitumor agents but the corresponding *trans* isomers are not. This is possibly due to the different manner in which these drugs attack DNA. There is considerable controversy in the literature over the type of DNA lesion caused by *cis*-platinum(II) complexes. Some have proposed an attack on a single guanine base via chelation to N(7) and O(6) as being the biologically important interaction. However, much indirect evidence suggests that binding to adjacent guanine bases in the same strand of DNA is important to the mechanism of action. Following initial binding to guanine bases, DNA is then locally denatured, exposing additional crosslinking sites. Thus, the selectivity of *cis*-platinum(II) complexes in inhibiting tumor growth may be due to a combination of intrastrand and interstrand crosslinking to DNA at areas of specific base sequences.

[Cancer Treat Rep 63:1445–1452, 1979]

The antitumor action of *cis*-dichlorodiammineplatinum(II) (*cis*-DDP) and its congeners (fig 1) appears to be related to a selective type of binding to replicating DNA. The studies discussed below reflect a growing consensus that DNA is the key target for *cis*-platinum drug action. Pascoe and Roberts (1) studied *cis*-DDP and *trans*-DDP in HeLa cells in suspension culture exposed to drug for 2 hours at 37°C. They compared the extent of binding of *cis*-DDP to various macromolecules in these tissue culture cells, and identified DNA as a preferred target (table 1). They found that on a dose basis, *cis*-DDP was 18.5 times more toxic than the *trans* isomer in assays of colony-forming ability. Drug binding to DNA in HeLa cells was correlated with the consequent decrease in cell survival. *cis*-DDP bound to DNA was more lethal. Thus, for 0.1 μmol of drug bound/g of cell DNA, *cis*-DDP caused > 90% decrease in cell survival, while *trans*-DDP had no significant effect. The interstrand crosslinking abilities of the two isomers were also compared. The number of interstrand and intrastrand crosslinks which must be introduced in each HeLa cell to reduce the survival fraction of cells to 37% is approximately equivalent: 350 for *cis*-DDP compared to 200 for *trans*-DDP. The extent of crosslinking for the same exposure of platinum drug was

TABLE 1.—Interactions of macromolecules and *cis*-DDP (*from ref 1*)

Macromolecule	Platinum bound per molecule*
DNA	22
mRNA	0.125
rRNA	0.033
tRNA	0.00067
Protein	0.00067

* Nucleic acids and protein were isolated by standard methods. Platinum bound to macromolecules was assayed by atomic absorption. Binding constants for each cellular component were determined and a comparison with component average molecular weights was made.

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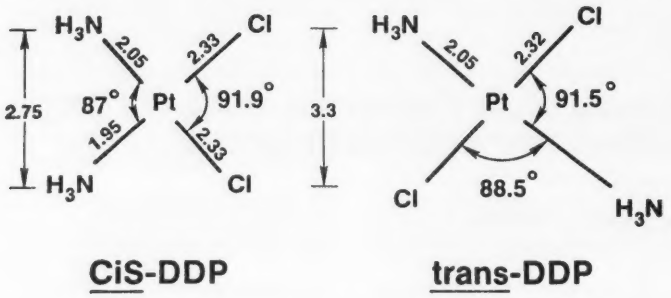

FIGURE 1.—Stereochemistry of the *cis* and *trans* isomers of DDP.

ten times greater with the *cis* than with the *trans* isomer. These studies suggest that interstrand crosslinking by platinum(II) drugs may be important and that the *cis* isomer is most effective in this damage. It is also clear that platinum(II) drug binding to DNA is not sufficient for cell lethality. Thus, cells can tolerate a considerable amount of *trans*-DDP bound to the DNA without lethality. In addition, more recently Fraval[4] has reported that stationary phase V-79 cells treated with *cis*-DDP have increased subculture cloning ability with time after drug treatment and removal. This correlates with a decrease in the amount of *cis*-DDP bound to DNA. Thus, for lethality, sufficient *cis*-platinum(II) must be bound to DNA during the active growth of cells. *cis*-DDP, in selectively impairing DNA replication, mimics the action of the bifunctional nitrogen mustards. The latter appear to exert biologic activity by crosslinking the complementary strands of DNA via the N(7) position of guanine (2). The alkylating agents have 8 Å separating their labile groups. Since the gap between the leaving groups on *cis*-DDP is only 3.3 Å (fig 1), this type of crosslinking should not occur. The proceedings of two recent conferences on *cis*-DDP and antitumor drugs contain much of the latest information on the importance of nucleic acid interactions (3).[5] The data are rather extensive and sometimes contradictory. Some reactions between platinum complexes and DNA bases have been studied in extremely nonphysiologic model test systems with such characteristics as low pH or high salt content, absence of chloride, or nonaqueous solvents. Interpretation of data from such experiments may not be applicable to tumor biology. We present our perspective of the chemically and biologically relevant interactions of *cis*-DDP with DNA to stimulate further definitive studies in this important field.

Chemistry of Platinum(II) Complexes

The majority of the antitumor complexes of platinum(II) exist in a square planar configuration (with four ligands in the same plane bound to platinum). The affinity of various ligands for platinum(II) decreases in the following order: CN^-, $:NH_3$, I^-, SCN^-:, Br^-, Cl^-, F^-, and H_2O) (4). Organic sulfur compounds are even more strongly bound. In *cis*-DDP, the chloride ligands are the groups which are replaced in biochemical reactions via

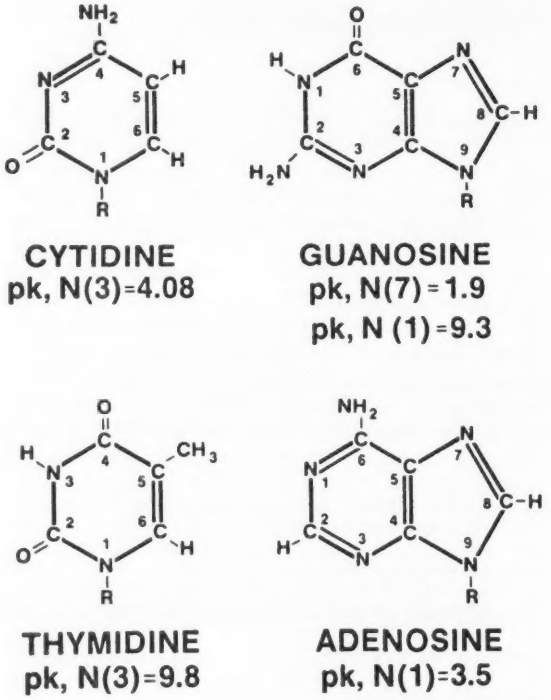

FIGURE 2.—Numbering systems for nucleosides found in DNA and their pKa's.

[4]Fraval HNA. Abstract presented at the EUCHEM Conference: Coordination Chemistry and Cancer Chemotherapy, Toulouse, France, 1978 (satellite symposium of the 7th International Congress of Pharmacology).

[5]EUCHEM Conference: Coordination Chemistry and Cancer Chemotherapy, Toulouse, France, 1978 (satellite symposium of the 7th International Congress of Pharmacology).

TABLE 2.—Relative No. of mols of hydrolyzed DEP ($PtCl_2en$)* (at pH 7.4) in a biologic system†

Species	Plasma	Cytoplasm
Cl^-	103 mmols	4 mmols
$PtCl_2en$	37.3	1.45
$[Pt(H_2O)Clen]^+$	1	1
$[Pt(H_2O)_2en]^{2+}$	1.34×10^{-3}	3.45×10^{-2}
PtCl(OH)en	1	1
$[Pt(H_2O)(OH)en]^+$	5.3×10^{-2}	1.38
$Pt(OH)_2en$	3.3×10^{-2}	0.86

*DEP is an effective antitumor analog of *cis*-DDP, in which the 2 ammonia groups are replaced by a single bridging ethylenediamine group.

†*From* Cleare MJ. J Clin Hematol Oncol, Part 1, vol 7:1–21, 1977.

a mechanism which involves partial replacement by water (aquation). The water itself can be hydrolyzed to $[H]^+$ and $[OH]^-$. The aquation chemistry of *cis*-DDP is important since the aquated products of *cis*-platinum(II) drugs are responsible for binding activity of the drug toward DNA. In addition, a *cis* configuration of two potential DNA binding sites is necessary for biologic activity. Ammonia groups are more strongly bound to platinum(II) and are not easily replaced. Relative amounts of aquated *cis*-DDP products expected to be present at equilibrium, in aqueous solution, are listed in table 2. The hydrolysis of *trans*-DDP occurs at a faster rate but its differences (in biologic activity) from *cis*-DDP are more likely of a structural nature.

Biologic Binding Sites for Platinum(II)

The purine and pyrimidine bases, deoxyribose sugars, and inorganic phosphate molecules of DNA contain potential ligands for binding to platinum. They are unimportant when stronger binding nucleophiles such as nitrogen are available, but may react if a situation for forming chelates exists (4). The DNA bases contain many ring nitrogen atoms with base pairs of electrons available for binding to platinum(II). Adenine, cytosine, and guanine also contain nitrogens in exocyclic amino groups, but their electrons are involved in delocalization with the ring systems and normally would not participate in platinum binding. Figure 2 identifies the numbering system used for purines and pyrimidines and their pKa's. Base preference for *cis*-platinum(II) was studied by Robins (5), using ^{14}C-labeled dichloroethylenediammineplatinum(II) (DEP), an active antitumor analog of *cis*-DDP. The kinetics of DEP binding were measured and it was found that guanine, adenine, cytosine, and their derivatives reacted with DEP but thymine did not. Guanine was the preferred base for interaction. Other studies using electronic absorption spectroscopy have dealt mainly with equilibrium conditions and confirm the importance of guanine in binding to platinum. Adenine also appears to be important since it can also be a preferred base for platinum binding (6).

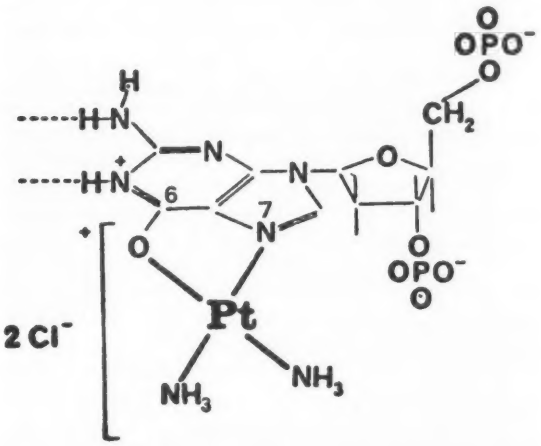

FIGURE 3.—Proposed method of binding of *cis*-DDP to guanine involving chelation to 1 purine base (9).

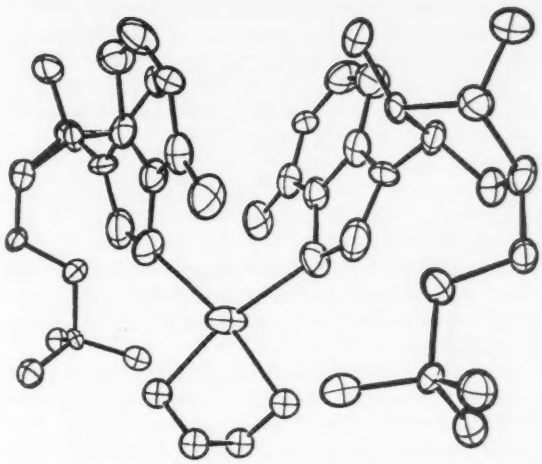

FIGURE 4.—Crystallographic structure of ethylenediammineplatinum bound via N(7) to adjacent purine nucleotides (10).

Kong and Theophanides (7) used ^{195}Pt-^1H spin-spin counting by nuclear magnetic resonance spectroscopy to show that mono- and bis-$[(NH_3)_2Pt]^{2+}$-inosine complexes were bound via the N(7) position. A study using proton and ^{13}C nuclear magnetic resonance spectroscopy to determine binding sites of the monofunctional ion $[Pt(H_2O)dien]^{2+}$ (where dien = diethylenetriamine) and bifunctional $[Pt(H_2O)_2en]^{2+}$ to uridine and adenosine was reported by Lim and Martin (8). There was a very slow reaction (2 days) with uridine at neutral pH values. This complex was very stable to replacement by excess chlo-

TABLE 3.—Crystal structure data on some complexes of guanine analogs with platinum amines (*from* ref 12)

cis-$[Pt(Gua)_2(NH_3)_2]^{2+}$	$[Pt(Gua)_2en]^{2+}$	*cis*-$Pt(5'\text{-}IMP)_2(NH_3)_2]^{2-}$
Pt-NH_3 = 2.079 Å	Pt-N(en) = 2.036 Å	Pt-NH_3 = 2.05 Å
Pt-NH_3 = 2.073 Å		
Pt-N(7) = 1.987 Å	Pt-N(7) = 1.967 Å	Pt-N(7) = 2.02 Å
Pt-N(7) = 2.038 Å	N(7)-Pt-N(7) = 87.8 Å	N(7)-Pt-N(7) = 89.8 Å

ride ion, suggesting the kinetic nature of chloride inhibition of platinum drug binding to nucleosides. Such slow binding is unlikely to be relevant to the antitumor activity of *cis*-DDP.

Two modes of *cis*-platinum(II) drug binding to DNA have been proposed to account for the antitumor activity of *cis*-DDP but not its *trans* isomer. One suggests that *cis*-DDP can bind to guanine bases via chelation to C(6)-O and N(7) as shown in figure 3 (9). The other proposes that the antitumor specificity of *cis*-platinum(II) drugs derives from a special affinity for adjacent guanine bases of DNA at N(7), as shown in figure 4 (10), and that resultant helix distortion and local melting allow interstrand crosslinks to form (11,12). We present below studies which might help discriminate between these two hypotheses.

Mansy and Tobias (13) investigated the reactions of nucleic acid components using, as references, perturbations to the Raman spectra which occur upon metallation at a single site. Inosine and its analogs were used as models for guanine-containing nucleosides. Complexes of the diaquo forms of *cis*-DDP and DEP were formed immediately on mixing with inosine, 1-methylinosine, and 5'-inosine monophosphate over a wide range of pH values. At pH values above 6, protons were found to be dissociated from N(1) and the pK of inosine was lowered from about 9.3 to 7.2 in the presence of an equimolar amount of *cis*-$[Pt(H_2O)_2(NH_3)_2]^{2+}$. The platinum drugs coordinated at both N(7) and N(1) of the base, with a polynuclear complex being formed. This excludes chelation via the C(6) oxygen and N(7) of inosine in the binding to platinum, because if this occurred no polynuclear complex could be formed. This view is supported by the data of Mansy and Tobias[6] showing that the carbonyl stretching frequency near 1700 cm^{-1} does not shift in the Raman spectra of aquated *cis*-DDP complexes of 5'-guanosine monophosphate.

The crystal structures of metal nucleoside complexes have been reviewed by Marzelli and Kistenmacher (14). They point out that N(7) is the binding site in all guanine complexes but the C(6)-O group may be involved in an inter-ligand hydrogen bond to a donor group on the other ligand. A number of crystallographic investigations have also been done on platinum complexes of nucleic acid residues and are summarized in a recent review (12). (By contrast, no reports have been made, to our knowledge, of a platinum anticancer drug bound to a purine via the C(6)-O and the N(7), suggesting that no such structures occur, or that they cannot be readily crystallized.) Table 3 provides the structural data on platinum-guanine-like complexes and supports a model of adjacent guanine bases linked via N(7) by the metal in platinum-DNA interactions.

A structure of a complex between platinum and an adenine derivative [(trichloro-9-methyladeninium)-platinum(II)] was reported recently by Terzis et al (15). There is no metal binding to this exocyclic amino group; the coordination is only through N(7). If binding to C(6)-NH_2 is important, one would have expected it to occur here. In a crystal structure of a dimethylsulfoxide-platinum complex, platinum binds to N(1) and N(7) of adenine-type bases (16). Cytosine complexes of platinum do form in solution and a structure of a 5'-cytosine monophosphate (CMP) complex has just been reported (17). This is unusual because two CMP molecules are linked by bridging ethylenediammineplatinum units with each platinum atom bound to an N(3) atom of one CMP and to an oxygen on the phosphate group of the other nucleotide. Such binding is unlikely in duplex DNA but it may be possible after denaturation has occurred as a result of other binding modes.

While studies with individual components of nucleic acids suggest preferential binding sites, reactions with polynucleotide macromolecules are of more significance. Since the N(3) site of cytosine [which can be attacked by platinum(II)] is involved in hydrogen bonding in double-stranded DNA, it is not generally considered readily available for platinum binding as long as the DNA is double-stranded. Thus, platinum(II) drug binding in the GpC base pairs is likely to occur through binding to guanine bases. Guanine bases possess N(7) and C(6)-O groups which are accessible for interaction with molecules that can fit in the major groove of DNA. In studies of chloride release (18) following mixture of *cis*-DDP with DNA, it was concluded that *cis*-DDP coordinated specifically to G-C planes of DNA. It was also found that two chloride ligands were released from *cis*-DDP for every drug molecule bound to the DNA. This suggested

[6]Mansy SA. Report presented at the 3rd International Symposium on Platinum Coordination Complexes in Cancer Chemotherapy, Dallas, Tex, 1977.

that the drug was being bound at two adjacent sites in DNA. These data were interpreted in favor of *cis*-DDP bound to N(7) and C(6)-O on guanine bases. We argue that these results are also consistent with *cis*-DDP bound at two adjacent N(7) sites in two neighboring guanine bases in the same DNA strand, since two chloride ions would be released from *cis*-DDP. Also, hydrolysis of *cis*-DDP can release chloride ions without interaction with DNA.

From proton-release measurements on DNA complexes (19), it was suggested that N(7) of guanine is the initial binding site for platinum with no proton release from DNA. In combination with chemical evidence, it was also suggested that protons are released in a second step which involves N(7) of adenine and N(1) of guanine with N(3) of cytidine. We find these studies difficult to evaluate since slow hydrolysis of *cis*-DDP and DEP releases protons as does DNA after interaction with platinum. The same group (20) has also studied electronic binding energies using X-ray photoelectron spectroscopy on solid DNA samples. They observed that the 1s binding energy of oxygen shifted to lower energy upon interaction of DNA with *cis*-DDP. Different results were obtained using *trans*-DDP, although identical effects were observed on the binding energy of nitrogen 1s electrons with both isomers. It is important to note that the binding energies are due to all the oxygen and nitrogen atoms in DNA and not just those on the guanine bases. Differing shifts in binding energies of DNA, nitrogen 1s for example, may be obtained from different charging conditions (12).

Evidence for Sequential Dependent Binding

The incremental buoyant density charges that occur as a result of reaction of DNA with *cis*-DDP were measured by Stone et al (21). Increase in buoyant density of DNAs treated with cis-DDP was directly proportional to the amount of *cis*-DDP bound. These studies also demonstrated that the amount of *cis*-platinum(II) bound was directly related to the G-C content of DNA. The effect of base sequence was then examined (11). Solutions of poly(dG)·poly(dC) or (poly dG·dC)^2 were each treated with *cis*-DDP in the presence of radiolabeled rat liver DNA. On the basis of G+C content alone, both G-C polymers were expected to exhibit a density increase only 2.4 times greater than that of mammalian DNA. However, the buoyant density of poly(dG)·poly(dC) increased 4.8 times over that of rat liver DNA, calf thymus DNA, or (poly dG·dC)^2. This suggested that sequential guanine bases, as in poly(dG)·poly(dC), are highly susceptible to attack by *cis*-DDP. Bidentate binding via N(7) to adjacent guanines in duplex DNA is plausible with *cis*-DDP but not with the *trans* isomer and could explain the unique antitumor effects of *cis*-platinum(II) drugs.

Our studies also indicated that *cis*-DDP does not bind to synthetic A+T-containing duplex polymers. By contrast, binding to such denatured polymers was readily demonstrated. We judged that binding to adenine in DNA is only favorable when the N(1) position of adenine is exposed. In double-stranded DNA molecules, this is involved in Watson-Crick hydrogen bonding to the thymine base. As has been demonstrated by earlier studies, the N(7) position on adenine is not a favorable binding site for platinum at pH 6.5. In natural DNAs, platinization of guanine bases and resultant local denaturation would permit binding of *cis*-DDP to adenine via N(1), to guanine via N(1), and to cytosine via N(3). More recent work by Munchausen and Rahn used luminescence properties, ultraviolet irradiation of platinized DNA, and viscosity measurements to determine effects of interaction with platinum complexes (22). Rates of reaction of platinized DNA with formaldehyde indicated that binding of *cis*-DDP to DNA does indeed induce small denatured regions.

Crystallographic studies show that when *cis*-platinum(II) drugs bridge at N(7), guanine base derivatives are tilted away from parallel alignments. Reactivity with many GpG sequences favors a mode of binding which would rapidly distort the duplex DNA and impede the formation of additional dimers. If C(6)-O and N(7) bidentate interactions were important, no differences would exist between the interactions of *cis*-DDP with poly(dG)·poly(dC) or (poly dG·dC)^2.

We have reviewed work of others showing G-C-dependent binding of *cis*-DDP to DNA (12). Munchausen (23) performed spectrophotometric titration of DNA with *cis*-DDP in which the binding capacity of *Haemophilus influenzae* DNA for *cis*-DDP was 12 platinum moieties/50 base pairs. By contrast, the binding capacity of the *trans* isomer was 22 platinum moieties/50 base pairs. Thus, bidentate binding of the *cis* isomer occurs whereas only monodentate binding of the *trans* complex is possible. She also showed that the extent of *cis*-DDP binding is dependent upon the G-C content of various DNAs.

If adjacent guanine bases are indeed linked by *cis*-platinum(II) drugs, the distance between bases, 3.4 Å, is reduced because the distance between two nitrogens coordinated to platinum is about 2.9 Å. This view is supported by the demonstration that ^{14}C-en-labeled DEP hinders the intercalation of 9-aminoacridine (9-AA), suggesting a compression to the helix because of intrastrand and/or interstrand crosslinking (24).

We studied the occurrence of intrastrand and interstrand crosslinks in DEP-platinized native DNA using restriction endonucleases (25). These studies demonstrate that the restriction endonuclease, Bam H1, maintains specific phosphodiester bond cutting in the presence of platinum bound to the DNA recognition sequence, 5′-GGATCC-3′. Separation of cleaved DNA into fragments

is blocked by platinum intrastrand or interstrand crosslinks. Recovery of specific fragments is achieved using dialysis with KCN after enzyme inactivation. Our data are consistent with data on GpG intrastrand bridging by platinum bound on either side of the cleavage site, and our previous work indicating a preference for binding to GpG sequences. This study also directs attention toward additional crosslink sites in the Bam H1 recognition sequence, 5′-GCATTCC-3′. Further work is in progress using enzymes which cut directly across both strands of duplex DNA at the recognition sites and yield "blunt" and not sticky ends. Preliminary results with the restriction endonuclease Hpa I, which recognizes the sequence 5′-GTTAAC-3′ and cleaves the phosphodiester bond between adenine and thymine in both complementary strands, confirm the occurrence of A-A interstrand crosslinks[7]; also, work with Hind III indicates that A-A intrastrand crosslinking by platinum does not readily occur (manuscript in preparation).

Studies by Jordan and Roos[8] using the 9-AA intercalation system and native DNAs of different base composition showed that crosslinking is preferred in GC-rich DNA sequences. With the duplex synthetic polymers (poly dA·dT)[2] an almost complete inhibition of 9-AA intercalation by DEP occurs. Since binding to thymine can be excluded, this also points to interstrand crosslinking by adenine bases on opposite strands. By contrast, with poly(dA)·poly(dT), 9-AA intercalation is unaltered, again ruling out intrastrand crosslinks between adjacent adenine bases. Similar work with (poly dG·dC)[2] showed considerable 9-AA inhibition, representing either G-C or C-C interstrand crosslinking or G-C intrastrand crosslinking.

Guanguli and Theophanide[9] also support the concept of increased crosslinking in DNA rich in G-C. They used a decrease in hyperchromic shift following DNA denaturation as an indicator of crosslinking. They interpret this finding as a bidentate mode binding of platinum to N(7) and C(6)-O of a single guanine resulting in breakage of hydrogen bonds and allowing interstrand crosslinks to occur. However, these data are entirely consistent with initial bridging of adjacent guanine bases by *cis*-platinum(II) with a resultant melting of *two* G-C base pairs and even more sites available for interstrand crosslinking.

Interstrand Crosslinking to DNA: Perhaps the Critical DNA Lesion in Animal Cells

It is established that *cis*-DDP forms interstrand crosslinks with DNA. Horacek and Drobnik, using ultraviolet spectroscopy, found that denaturation of double-stranded DNA was inhibited by high ratios of platinum/DNA-phosphate, presumably as a result of crosslinking (26). Further elegant evidence for crosslinking has been obtained in a number of experiments by Pascoe and Roberts (1) using BUDR substituted in one strand of duplex DNA as a density label. Crosslinked DNA appears as an intermediate peak in isopycnic salt gradients in alkaline conditions. They have found differing extents of crosslinking of DNA by *cis*-DDP and *trans*-DDP in vitro and in vivo.

Munchausen and Rahn (22) studied DNA crosslinking by measuring the resistance of radioactively labeled DNA to single-strand-specific nuclease after heat denaturation and rapid cooling. Only crosslinked molecules re-anneal and remain undigested. Their work on the effect of *cis*-DDP on the transforming ability of *H. influenzae* DNA demonstrated that *cis*-platinum prevented transformation of a platinum:base phosphate molar ratio of only 0.001 (23). Since *cis*-DDP is a biologically inactivating species at only 0.1% of saturation of the DNA bases, while the first crosslink occurs (per 1000 bases) when about 20 platinum molecules are bound to 1000 bases, ie, the DNA is 2% saturated, crosslinking seems unimportant.

The suppression of transformation by doses of *cis*-platinum(II) which cause rare interstrand crosslinks per DNA molecule has indicated, to some, a minor role of such crosslinks in the antitumor activity of *cis*-DDP. This assay depends on uptake of naked DNA into bacteria deficient in a single gene. An interstrand crosslink is unlikely to be important in the uptake of exogenous DNA because statistically few crosslinks would occur in any particular gene. Platinization of DNA, even at very low levels, will cause many local distortions and thus renders the DNA more recognizable for destruction and prevents transformation. However, mammalian chromosomes are much more complicated, possessing higher structure with histone and nonhistone proteins. In vivo, damaged individual bases may be readily excised and repaired. The total DNA content of the mammalian cell has to be capable of being replicated without loss of host chromosomes. Thus, one cannot exclude a critical role of interstrand crosslinking in the antitumor activity of *cis*-DDP by such studies with uptake of naked DNA in bacteria.

[7]Kelman AD. Report presented at the EUCHEM Conference: Coordination Chemistry and Cancer Chemotherapy, Toulouse, France, 1978 (satellite symposium of the 7th International Congress of Pharmacology).

[8]Jordan DO, and Roos IAG. Abstract presented at the EUCHEM Conference: Coordination Chemistry and Cancer Chemotherapy, Toulouse, France, 1978 (satellite symposium of the 7th International Congress of Pharmacology).

[9]Guanguli PK, and Theophanides T. Abstract presented at the EUCHEM Conference: Coordination Chemistry and Cancer Chemotherapy, Toulouse, France, 1978 (satellite symposium of the 7th International Congress of Pharmacology).

TABLE 4.—Frequencies of nearest neighbor base sequences in DNA from various sources*

DNA	% G-C	% d(GpG)
Mycococcus luteus	72	5.6
Mycobacterium phlei	68	4.5
Aerobacter aerogenes	55	3.3
Escherichia coli	50	2.8
Bacillus subtilis	44	2.3
Calf thymus	43	2.5

**From* Josse J, Kaiser AD, and Kornberg A. J Biol Chem 236:834, 1961.

Conclusion

Most antitumor platinum drugs to date have had two relatively labile groups, mainly chloride ligands in positions *cis* to one another, and have been square planar and neutral. These properties permit facile transport through biologic membranes, and significant replacement of chloride by water would not be expected to take place until the drug reached the intracellular regions.

The interactions of *cis*-DDP and analogs with DNA both in vitro and in vivo are probably indicative of important biologic mechanisms. The *cis* complexes may bind in a monofunctional or bidentate fashion to DNA, whereas the *trans* isomers may bind only monofunctionally because of structural factors. Subsequently, denatured (ie, open-looped) DNA provides additional binding sites with new structural possibilities. The ammine groups on the platinum drugs are important in stabilizing DNA interactions by hydrogen bonding effects.

The following model for the binding of *cis*-DDP to DNA may account for the antitumor action of *cis*-platinum(II) drugs. The first site of attack is at N(7) of a guanine base in the major groove of DNA. The platinum complex is probably partially hydrolyzed via $[cis\text{-}PtCl(H_2O)(NH_3)_2]^+$. Immediately, there is disruption of the hydrogen bonding between guanine and its associated cytosine base.

According to our mode, the next mode of binding occurs to an adjacent guanine base to form an intrastrand crosslink (after loss of the chloride ligand), and the guanine base is tilted out of planarity with other bases in the same strand. If no adjacent guanine base exists, the platinum complex is probably bound only very weakly and may be removed in situ, because of unfavorable equilibrium conditions. Other workers suggest instead that chelation to single guanine bases of N(7) and C(6)-O occurs. Further work is required to discriminate between these two proposed bidentate lesions involving *cis*-platinum(II) and guanine bases.

After binding to guanine bases occurs, local perturbation and distortion of the DNA double helix must take place with resultant local denaturation. This exposes sites on the adjacent DNA strand for interstrand crosslinking. The adjacent guanine bases could be clipped together via binding to N(7) atoms alone on different bases or to C(6)-O atoms on different bases. We prefer the N(7) mode of interaction for *cis*-DDP based on solution and crystal structure data. Since the amount of GpG sequences in DNA is rather low, only a small amount of *cis*-DDP would react in vivo with DNA. Table 4 lists the percentage of GpG sequences in DNA from various sources and shows an increase with G-C content. We have previously presented the theory that *cis*-DDP selectively binds to adjacent guanine bases in DNA (11,12). With our current data, indicating that *cis*-platinum(II) interstrand crosslinks occur between certain bases only, in natural DNA, we now extend our model: we suggest that DNA with ApT or TpA sequences adjacent to GpG would be especially sensitive to interstrand crosslinks between adenine bases on opposite strands.

The bound *cis*-platinum(II) drug would result in template inactivation of DNA and the accumulation of potentially lethal damage. It is of interest that Harder and Graham Smith (27) found that A-A interstrand crosslinks in synthetic DNA were extremely effective in blocking template function. Current work in progress in several laboratories should further clarify the role of particular base sequences in the biologic activity of *cis*-platinum(II) anticancer drugs.

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Antitumor Activity of *cis*-Dichlorodiammineplatinum(II)

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SUMMARY

***cis*-Dichlorodiammineplatinum(II) has shown good broad-spectrum activity in the current Division of Cancer Treatment (National Cancer Institute) tumor panel. It meets Decision Network criteria for activity in at least five of ten tumor systems (the ip B16 melanoma, the sc $CD8F_1$ mammary carcinoma, the ip colon tumor 26, the ip L1210 leukemia, and the ip P388 leukemia). Activity against the sc colon tumor 38, the iv Lewis lung tumor, the ic ependymoblastoma, and the human breast tumor xenograft (MX-1) was marginal. No activity was detected against the human lung tumor xenograft (LX-1), but good, reproducible activity was observed against the murine M5076 ovarian carcinoma. No schedule-dependency was observed after ip administration against the ip L1210 leukemia. The recently developed subrenal capsule assay offers promise as a rapid way (≤ 11 days) of ranking the sensitivity of a variety of human tumors to *cis*-dichlorodiammine-platinum(II) and other chemotherapeutic agents.**

[Cancer Treat Rep 63:1453–1458, 1979]

After the initial observation by Rosenberg and coworkers in 1965 that certain coordination compounds of platinum inhibited bacterial replication (1) and their later finding in 1969 that several of these complexes also had significant antitumor activity (2), intense interest in the biologic properties of platinum complexes began to emerge. Of the complexes they described, *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) appeared to be one of the most promising for development to clinical trial. Since that time, a number of reports have appeared on the antitumor activity of *cis*-platinum and related congeners against L1210 leukemia and other transplantable rodent tumors (3–7).

The Division of Cancer Treatment (DCT) of the National Cancer Institute (Bethesda, Md) has also examined *cis*-platinum extensively in a variety of animal tumor systems, including the tumor panel which was adopted in November 1975 by the Developmental Therapeutics Program (DCT, National Cancer Institute) as the standard screen for new materials and as an aid to the selection of the most active compounds among a series of structural congeners. The objective of the screen is to test about 500–1000 compounds per year against a panel of murine tumors, with particular emphasis on solid tumors which represent a histologic counterpart of the most common forms of human cancer. In addition to mouse colon, breast, and lung (Lewis lung) tumors, as well as the more traditional B16 melanoma and P388 and L1210 leukemias, the panel also includes human tumor xenografts of colon, breast, and lung carcinomas which are maintained by serial transplant in nude (athymic) mice, as previously described (8,9).

The animal test results from the complete panel will be used as a major selection criterion for new materials entering clinical trial and also as a data base for future animal-man clinical correlation studies.

Antitumor Activity of *cis*-Platinum

Using a recently devised computer program, table 1 shows the average historical responses of *cis*-platinum in a variety of murine tumor systems maintained by the DCT. These results indicate that *cis*-platinum has good broad-spectrum activity, with marked, reproducible activity against the ip B16 melanoma, the sc $CD8F_1$ mammary carcinoma, the ip colon tumor 26, and the ip L1210 and P388 leukemias. Activity against the sc colon tumor

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TABLE 1.—Historical antitumor activity of *cis*-platinum against murine systems

System	Optimal dose range (mg/kg)	Schedule	No. of trials	% T/C*		Score†
				Range	Mean ± SE	
B16 melanoma (ip)	0.15–20	qd × 9 ip	598	110–289	178 ± 1.2	+ +
$CD8F_1$ mammary carcinoma (sc)	4–12.5	q7days × 5 ip	7	4–0‡	1 ± 0.6‡	+ +
Colon tumor 26 (ip)	2–4	q4days × 2 ip	3	195–263	228 ± 19.6	+ +
Colon tumor 38 (sc)	4–16	q7days × 2 ip	5	46–11‡	30 ± 8.0‡	+
L1210 leukemia (ip)	0.25–4	qd × 9 ip	136	101–207	158 ± 2.2	+ +
Lewis lung tumor (sc)	0.5–2	qd × 9 ip	3	107–142	122 ± 10.4	–
Lewis lung tumor (iv)	0.5–2	qd × 9 ip	22	100–238	154 ± 6.7	+
P388 leukemia (ip)	0.25–2.5	qd × 9 ip	82	121–353	225 ± 4.3	+ +
Ependymoblastoma (ic)	0.8–2	qd × 5 ip	18	101–169	133 ± 4.4	+

*Based on median survival time or tumor size (‡).

†+ + = meets Decision Network criteria for activity; + = reproducible activity; – = inactive. Testing was performed according to the protocols of the Drug Evaluation Branch (ref 15). Tumors were implanted on Day 0 and drug injections were started on Day 1.

38, the iv Lewis lung tumor, and the ic ependymoblastoma was reproducible, but marginal.

Schedule-Dependency Testing

Table 2 summarizes the effect of altering both the schedule and the route of administration on the activity of *cis*-platinum against ip L1210 leukemia. *cis*-Platinum was active on all schedules of ip administration and, therefore, would not be considered to be schedule-dependent for activity. Whether changing the schedule of administration affected the toxicity of *cis*-platinum was not examined in these studies. *cis*-Platinum was most active when given ip against an ip implanted tumor; its activity declined after either sc or iv administration. No activity was detected after oral administration.

Activity of *cis*-Platinum Compared to Other Agents

cis-Platinum compares favorably in activity with other clinically active agents against the spectrum of murine tumors which comprise the tumor panel (table 3). With the exception of methotrexate, most of the clinically active agents, such as 5-fluorouracil (5-FU), cyclophosphamide, and Adriamycin, have good broad-spectrum activity. By contrast, many of the agents introduced into clinical trial since 1970, such as hexamethylmelamine, cytembena, 3-deazauridine, triazinate (Baker's antifol), pyrazoloimidazole, and pentamethylmelamine, have little or no activity in four or more systems in the tumor panel (table 4).

Other agents which, like *cis*-platinum, have high ac-

TABLE 2.—Effect of schedule and route of administration on activity of *cis*-platinum against L1210 leukemia ip

Schedule	Route	Optimal dose range (mg/kg)	No. of trials	% T/C*	
				Range	Mean ± SE
qd × 1	ip	1–20	34	102–378	183 ± 11.0
qd × 5	ip	1–4	13	125–217	174 ± 7.0
qd × 5	sc	2	1	143	143
qd × 5	iv	2	1	122	122
qd × 5	oral	4	1	< 120	< 120
qd × 9	ip	0.15–4	164	101–207	158 ± 1.9
qd × 9	oral	8	1	120	120
q3hrs × 8 on Days 1 and 9 (16)†	ip	2	1	196	196
q3hrs × 8 on Days 1, 5, and 9 (24)†	ip	1	1	206	206
q4days × 3	ip	1–10	229	114–381	167 ± 2.1
q4days × 3	sc	2.5	1	127	127
q4days × 3	oral	8	1	< 120	< 120

*Based on median survival time. Tumor was implanted on Day 0 and drug injections were started on Day 1; % T/C ≤ 120 is considered inactive.

†Nos. in parentheses = Total No. of injections.

TABLE 3.—Comparison of the activity of *cis*-platinum in the tumor panel with other clinically active agents

Drug (ip)	Scores* for— Mouse tumors: B16 melanoma (ip)	$CD8F_1$ mammary carcinoma (sc)	Colon tumor 26 (ip)	Colon tumor 38 (sc)	L1210 leukemia (ip)	Lewis lung tumor (iv)	P388 leukemia (ip)	Human tumor xenografts†: MX-1 breast (sc)	CX-1 colon (sc)	LX-1 lung (sc)
cis-Platinum	+ +	+ +	+ +	+	+ +	+	+ +	+	NT	–
Methotrexate	–	–	–	–	+ +	–	+ +	+–	NT	–
6-Mercaptopurine	–	+	+ +	+	+ +	–	+	–	NT	–
Melphalan	+ +	+ +	+ +	+	+ +	+	+ +	+ +	NT	+
5-FU	+	+ +	+	+ +	+ +	–	+ +	+–	+	–
Cyclophosphamide	+	+ +	+ +	+ +	+ +	+ +	+ +	+ +	NT	–
Vincristine	+ +	+ +	–	–	+	–	+ +	+ +	NT	–
Adriamycin	+ +	+ +	+ +	–	+ +	+–	+ +	–	NT	–
BCNU	+ +	+	+ +	+–	+ +	+ +	+ +	+	NT	IT
Actinomycin D	+ +	+ +	+	+	+	–	+ +	+	NT	+

*+ + = meets Decision Network criteria for activity; + = active; +– = erratic, retest; – = inactive; NT = not tested; IT = incomplete test. Data summarized were from the following routine drug schedules: B16, qd × 9; $CD8F_1$, q7days × 5; colon 26, q4days × 2; colon 38, q7days × 2; L1210, qd × 9; Lewis lung, qd × 9; P388, qd × 9; MX-1 breast, q4days × 3 or q7days × 4; CX-1 colon, q4days × 3 or q7days × 4; and LX-1 lung, q4days × 3 or q7days × 4. Tumors were implanted on Day 0 and drug injections were started on Day 1, except for xenograft testing, where drug injections were delayed until the median tumor weights were in the 100–300-mg range.

†Data of Battelle Memorial Institute (Columbus, Ohio).

tivity in four or more tumor panel systems include the nitrosoureas, ifosfamide, ICRF-159, dianhydrogalactitol, piperazinedione, VP-16-213, mitomycin C, neocarzinostatin, PALA, and AMSA. Whether activity in a broad spectrum of animal tumors or in a particular tumor type is more important as a criterion for selecting agents for clinical trial is not known at this time. Data from the human tumor xenograft testing are still too meager for any definitive conclusions about the potential usefulness of these systems in finding new agents for clinical development.

Of considerable interest is the recent finding that the murine M5076 ovarian carcinoma responds well to both *cis*-platinum and hexamethylmelamine, two agents being used clinically for the treatment of ovarian cancer (10) (table 5). The M5076 tumor arose spontaneously in a C57BL mouse in the laboratory of W. F. Dunning of the Papanicolaou Research Institute (Miami, Fla) and has been developed as a screening system by Arthur Bogden of the Mason Research Institute (Worcester, Mass).

Using the technique of carcinogen induction, Thomas Corbett of the Southern Research Institute (Birmingham, Ala) has succeeded in developing a series of transplantable mouse colon tumors with different biochemical, histologic, and growth characteristics as well as different responses to drugs (11,12). Table 6 summarizes the antitumor activity of selected agents, including *cis*-platinum, against a panel of six tumors. Although *cis*-platinum was not highly active against any of the tumors, at least marginal to moderate activity was observed against all of the tumors. 5-FU, a commonly used agent for the treatment of human colon cancer (13), was highly active only in colon tumor 38 and totally inactive in colon tumor 06/A.

These vast differences in drug responsiveness illustrate the difficulties in selecting *one* animal colon tumor to screen for new drugs for all types of human colon cancer. These results also point out the fallacy of the common statement that "5-FU is active against colon cancer," for some tumors respond and others do not.

Subrenal Capsule Technique

Recently, Arthur Bogden of the Mason Research Institute developed an 11-day subrenal capsule assay in athymic nude mice which can be used for ranking drug activities using a variety of human tumors (14).[2] More recently, he has found that similar results can be obtained in only 6 days using conventional BDF_1 mice.[3,4] A 6-day

[2]Bogden AE, and Kelton DE. Development of new in vivo tumor systems. Annual Progress Report to the National Cancer Institute for June 3, 1976, to June 2, 1977, contract N01-CM-57030, Mason Research Institute, Worcester, Mass.

[3]Bogden AE, and Kelton DE. Development of new in vivo tumor systems. Annual Progress Report for June 3, 1977, to June 2, 1978, contract N01-CM-57030, Mason Research Institute, Worcester, Mass.

[4]Bogden AE, Haskell PH, LePage DJ, et al. An in vivo method for predicting the response of individual human tumors to specific chemotherapeutic agents: subrenal capsule assay. Manuscript in preparation.

TABLE 4.—Comparison of the activity of *cis*-platinum in the tumor panel with selected agents introduced into clinical trial since 1970

Drug (ip)	Scores* for—									
	Mouse tumors							Human tumor xenografts†		
	B16 melanoma (ip)	$CD8F_1$ mammary carcinoma (sc)	Colon tumor 26 (ip)	Colon tumor 38 (sc)	L1210 leukemia (ip)	Lewis lung tumor (iv)	P388 leukemia (ip)	MX-1 breast (sc)	CX-1 colon (sc)	LX-1 lung (sc)
cis-Platinum	+ +	+ +	+ +	+	+ +	+	+ +	+	NT	–
6-Thioguanine	–	+ +	+ +	+	+ +	–	+	–	NT	–
Hexamethylmelamine	–	+	–	–	–	–	–	+	–	–
CCNU	+ +	+	+ +	+ +	+ +	+ +	+ +	NT	–	+
Methyl-CCNU	+ +	+ +	+ +	+ +	+ +	+ +	+ +	NT	–	+
5-Azacytidine	+	+ +	+	+	+ +	+	+ +	–	NT	–
Dibromodulcitol	–	+ +	+	+ +	+	NT	+	NT	NT	NT
Cytembena	–	–	–	–	–	–	+	NT	NT	NT
Ifosfamide	+	+ +	+	+	+ +	+ +	+ +	NT	NT	NT
3-Deazauridine	–	–	–	–	+	–	–	NT	NT	NT
ICRF-159	+	+ +	+	+	+ +	+ +	+ +	NT	NT	NT
Dianhydrogalactitol	+ +	+ +	+ +	+	+ +	NT	+ +	NT	NT	NT
Piperazinedione	+	+ +	+ +	+	+ +	–	+ +	+ +	NT	–
Triazinate (Baker's antifol)	–	–	–	–	–	NT	–	–	NT	NT
Anguidine	+	+	–	+	+	–	+ +	–	–	–
VP-16-213	+ +	+ +	+ +	+	+ +	NT	+ +	NT	NT	NT
Mitomycin C	+ +	+ +	+	+ +	+ +	NT	+ +	+ +	NT	NT
Hycanthone	–	+	–	+	+	NT	+	NT	NT	NT
Pyrazofurin	–	+	–	+–	+	NT	+	NT	NT	NT
Cyclocytidine	+	+	+–	+	+ +	+ +	+ +	NT	NT	NT
Ftorafur	–	+ +	–	+–	+	–	+	NT	NT	NT
Neocarzinostatin	+ +	+–	+ +	–	+ +	–	+ +	NT	NT	NT
Rubidazone	+ +	–	+	+–	+	–	+ +	NT	NT	NT
Soluble ICRF-159	+	+	+	+	+ +	+	+ +	NT	NT	NT
Chlorozotocin	+ +	+	+ +	+	+ +	–	+ +	+	NT	NT
Pyrazoloimidazole	–	–	–	–	+	+–	–	NT	NT	NT
PCNU	+ +	+ +	+ +	+ +	+ +	+ +	+ +	NT	NT	NT
Pentamethylmelamine	–	+–	–	+	–	–	–	+	–	–
Indicine-*N*-oxide	+ +	+	+–	–	+	–	+ +	+	NT	NT
Bruceantin	+ +	–	–	+–	+	–	+ +	–	NT	NT
PALA	+ +	+ +	+ +	+	–	+ +	+	+	NT	–
AMSA	+ +	+ +	+–	–	+ +	–	+ +	–	NT	NT

*See table 3 for scoring system.
†Data from Battelle Memorial Institute.

assay was selected because the humoral response to a primary antigenic stimulus in a normal mouse generally evolves in 7–10 days and cell-mediated responses to an initial allograft become evident in 9–12 days. Briefly, the technique involves implanting a 1-mm^3 tumor fragment under the kidney capsule and measuring the size in situ with the aid of a stereoscopic microscope equipped with an ocular micrometer. Chemotherapy is then initiated using one or more doses and schedules of administration. The assay is terminated by killing the rat, the tumor-bearing kidneys are excised, and the final in situ tumor size is determined. Body weights are determined at the beginning and end of the assay as a measure of drug toxicity. Tumor response is recorded as a change in tumor size in ocular micrometer units during the period of assay. The assumption is made that a tumor increases in size when cell division exceeds cell death, decreases in size when cell death exceeds cell division, and remains stationary when cell division and cell death are equal.

Table 7 compares typical responses of several clinically active agents in the subrenal capsule assay in both normal BDF_1 and athymic nude mice with responses obtained in

TABLE 5.—Response of murine M5076 ovarian carcinoma to agents of clinical interest in ovarian cancer*

Drug	Response†
cis-Platinum	+ +
Methotrexate	–
Actinomycin D	–
Melphalan	+ +
Hexamethylmelamine	+ +
5-FU	–
Cyclophosphamide	+ +
Adriamycin	–

*Data from the Mason Research Institute.

†Evaluation criteria based on mean tumor size on day of evaluation: – = % T/C, > 40; + = % T/C, 21–40; + + = % T/C, 0–20.

the subcutaneous assays. Although the correlations were fairly good, they were not perfect, possibly because of differences in routes and schedules of drug administration. For example, Adriamycin was highly active sc on a daily × 10 schedule against the MX-1 tumor using the subrenal capsule assay in athymic nude mice but was inactive iv on an every 7th day × 4 schedule in the subcutaneous assay. However, the fact that this technique can potentially rank several drugs in 6 days using a variety of human tumors[4] is an attractive feature which merits further study. It represents a potentially feasible way to help reduce the element of chance in selecting therapy for an individual patient.

TABLE 6.—Antitumor activity of selected agents against 6 transplantable mouse colon tumors*

Drug	Score† for mouse colon tumor—					
	26	36	38	51	06/A	07/A
cis-Platinum	+ + +	+ + +	+ +	+ +	+	+
Methotrexate	–	–	–	–	–	
5-FU	+ + +	+ +	+ + + +	+	–	+ + +
Cyclophosphamide	+ +	+ + +	+ +	+ +	+ + + +	
Vincristine	–	+	+	–	–	±
Adriamycin	+ +	+ +	+ +	+ +	+ +	±
BCNU	+ + + +	+ + +		+		
Anguidine	–	–	+ + + +	–	+ + +	
AMSA		–	–			–
PALA	+	–	+ +	+	–	
Bruceantin	–	+		–		
PCNU	+ + + +		–	+		+
Pyrazoloimidazole	–	+ + +	–			
Indicine-*N*-oxide	–	+	–			

*Data from the Southern Research Institute. All tests were carried out against sc implanted tumors, except for colon tumor 26, which was implanted ip.

†Scoring system:

Activity rating		Single-course therapy, T-C value (a) (days)	Multiple weekly courses (≥ 3), T-C value (days)	Single-course therapy tumor measurements, T/C value (%)	Single-course lifespan assay (% increase in lifespan)
Highly active (b)	+ + + +	> 19	> 31	0	> 110
	+ + +	13–19	22–31	1–10	78–110
	+ +	9–12	15–21	11–24	46–77
Marginally active	+	7–8	10–14	25–42	30–45
Inactive	–	< 7	< 10	> 42	< 30

a: time (days) for treated tumors to reach 750–1250 mg minus time (days) for untreated control tumors to reach the same size; 750-mg size is used when treatment is started shortly after tumor implant and 1250-mg size is used when tumors are established and advanced (about 500 mg) at start of drug treatment.

b: no agent is ranked as + + + + unless the lifespan increase is ≥ 40% in addition to the required T-C value.

TABLE 7.—Responsiveness of human tumors in subrenal capsule (normal BDF_1 and athymic nude mice) and subcutaneous (athymic nude mice) assays

Drug	Subrenal capsule*		Subcutaneous†
	Normal BDF_1	Athymic nude	Athymic nude
MX-1 human breast tumor			
Melphalan	+ +‡	+ +	+ +
Cyclophosphamide	+ +	+ +	+ +
Methotrexate	+	+ +	+ –
5-FU	+ +	+	+ –
Adriamycin	+	+ +	–
Methyl-CCNU	+ +	+	NT
cis-Platinum	NT	+ +	+
LX-1 human lung tumor			
Cyclophosphamide	–	–	–
Adriamycin	–	–	–

*Data from Mason Research Institute.

†Data from Battelle Memorial Institute.

‡Scoring system: NT = not tested; + – = erratic, retest. For subrenal capsule assay: + + = elicits tumor regression (T/C < 1%), + = retards rate of tumor growth (T/C ≤ 30%), – = inactive (T/C > 30%). For subcutaneous assay: + + = elicits tumor regression (relative tumor weight [RW] < 1), + = retards rate of tumor growth (TRW/CRW = ≤ 42%), – = inactive (TRW/CRW = > 42%).

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cis-Dichlorodiammineplatinum(II): Combination Chemotherapy and Cross-Resistance Studies With Tumors of Mice [1]

F. M. Schabel, Jr,* M. W. Trader, W. R. Laster, Jr, T. H. Corbett, and D. P. Griswold, Jr [2,3]

SUMMARY

***cis*-Dichlorodiammineplatinum(II) (*cis*-platinum) has no more than additive, and often much less than additive, lethal toxicity for mice when given in combination with other anticancer agents representing several of the major functional classes of clinically useful anticancer drugs. The previously reported broad spectrum of anticancer activity of *cis*-platinum against tumors in laboratory animals has now been extended to promisingly useful therapeutic synergism in combination with other active anticancer drugs, including advanced-staged tumors in mice; eg, *cis*-platinum plus cyclophosphamide against advanced Ridgway osteogenic sarcoma and advanced P388 leukemia, and as surgical adjuvant chemotherapy against advanced colon tumor 26; *cis*-platinum plus Adriamycin against advanced P388; and *cis*-platinum plus VP-16-213 against advanced P388. Therapeutic synergism was also seen with *cis*-platinum plus carminomycin (an Adriamycin analog) against early colon tumor 26. Resistance and cross-resistance studies using sublines of L1210 and P388 selected for resistance to various alkylating agents (cyclophosphamide, melphalan, BCNU, or *cis*-platinum) indicate a variety of resistance and cross-resistance patterns which further support the growing body of evidence that wide differences in mechanism of cytotoxic activity exist among alkylating agents having experimentally and clinically useful anticancer activity. These data support the observed therapeutic synergisms with combinations of alkylating agents seen against a broad spectrum of murine tumors, and they suggest other drug combinations that might be considered for experimental and clinical trial based on a growing number of logical differences in biochemical mechanism of action of alkylating agent anticancer drugs that have been reported.**

[Cancer Treat Rep 63:1459–1473, 1979]

The report by Rosenberg et al (1) that *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) had therapeutic activity against animal tumors was an important discovery because the platinum complexes represented a new chemical class of anticancer drugs. Further study established that *cis*-platinum had broad-spectrum anticancer activity in animals which made it even more promising for consideration for practical application. [4]

Combination chemotherapy, a common practice in clinical oncology, is used with the hope that one or more of the assets needed by drug combinations to show therapeutic synergism [5] will be present in the combination.

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[4]Published reports indicate that *cis*-platinum has carcinostatic or cytotoxic activity in vivo against at least 18 different sarcomas, carcinomas, and leukemia-lymphomas of mice, rats, or hamsters representing the major histologic and organ types of cancer in man.

[5]Therapeutic synergism = a greater therapeutic effect against the tumor by therapeutically acceptable doses (≤ LD10 in animals) of the drugs in combination than can be obtained with acceptable doses of any component(s) of the drug combination.

TABLE 1.—Combination toxicity index of *cis*-platinum + selected agents

Agents	Strain of mouse used	Route and schedule	Combination toxicity index
cis-Platinum + CPA	CDF_1	ip, single dose	1.1
	BDF_1	*cis*-Platinum, iv + CPA, ip } q7days × 3	1.2
cis-Platinum + melphalan	CDF_1	ip, single dose	ca 2.0
cis-Platinum + BCNU	CDF_1	ip, single dose	1.7
cis-Platinum + methyl-CCNU	CDF_1	*cis*-Platinum, iv + Methyl-CCNU, oral } q7days × 3	0.86
cis-Platinum + ADR	BDF_1	ip, single dose	ca 2.0
		ip, q4days × 3	ca 1.9
		ip, qd, 1–9 days	1.3
cis-Platinum + carminomycin	CDF_1	iv, q4days × 3	1.2
cis-Platinum + DIOH-AA*	BDF_1	iv, q2days × 6	1.5
cis-Platinum + 5-FU	BDF_1	ip, qd, 1–5 days	1.5
cis-Platinum + VP-16-213	BDF_1	ip, q4days × 3	> 1.3

*9,10-Anthracenedione, 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-.

These assets are (*a*) that the drugs used in the combination will be less than additive in toxicity for vital normal cells, (*b*) that the drugs will have different biochemical mechanisms of cytotoxic activity for drug-sensitive tumor cells, and/or (*c*) that tumor cells resistant to one or more drugs in the drug combination used in treatment will be sensitive to one or more other drugs in the combination. All three of these therapeutic assets are known to obtain with a number of clinically useful drug combinations and are always sought with new drug combinations.

TOXICITY FOR MICE TREATED WITH *cis*-PLATINUM IN COMBINATION WITH OTHER ANTICANCER DRUGS

Skipper (2) has reported a method for quantitatively determining the lethal toxicity of combinations of two drugs for mice. Briefly, his procedure is as follows: (*a*) the doses of individual drugs used in a combination toxicity study are tabulated as decimal fractions of the previously determined LD10s for the same treatment schedule, and (*b*) the decimal fraction of the LD10s of the individual drugs used in the drug combination are added and the sums are plotted against observed mortality on log-probit paper. The LD10 (sum) of the combination is read from a line of best fit. He has identified the drug combination LD10 determined in this manner as the combination toxicity index (CTI). Illustrative plots using Skipper's method are shown for *cis*-platinum plus Adriamycin (ADR) in figure 1 and for *cis*-platinum plus 5-fluorouracil (5-FU) in figure 2. A CTI of about 1 indicates additive toxicity for vital normal cells, a CTI of < 1 indicates greater than additive toxicity, and a CTI of > 1 indicates less than additive toxicity. In table 1 we have listed the CTIs of a number of drugs, representing different functional classes of anticancer agents, in combination with *cis*-platinum, using a number of different treatment schedules. Data presented in table 1 indicate that: (*a*) *cis*-platinum appears to be additive in toxicity in single-dose treatment in combination with cyclophosphamide (CPA) but less than additive in single-dose treatment with melphalan or BCNU, (*b*) combination toxicity of *cis*-platinum plus ADR appears to be schedule dependent (the CTI increases as the interval between doses increases), (*c*) the CTI of *cis*-platinum plus ADR is higher than that of *cis*-platinum plus carminomycin (an ADR analog which is considerably more toxic on a molar basis than ADR), and (*d*) *cis*-platinum is less than additive in toxicity in combination with at least some alkylating agents, DNA binders or intercalators, and antimetabolite anticancer agents. These data indicate that *cis*-platinum probably possesses the first asset for combination chemotherapy: less than additive toxicity for vital normal cells when used in combination with representatives of the other major functional classes of anticancer drugs.

CHEMOTHERAPY OF ANIMAL TUMORS WITH *cis*-PLATINUM IN COMBINATION WITH OTHER ANTICANCER AGENTS

Many drug combinations containing *cis*-platinum have been tested against murine tumors and a number of drug combinations have been reported to be therapeutically synergistic. We have listed the published reports as a limited summary of previous work and have included the

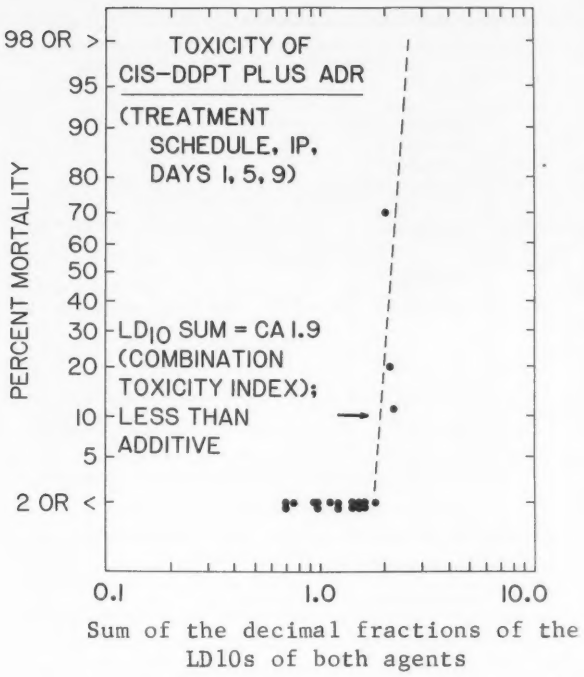

FIGURE 1.—Combination toxicity index of *cis*-platinum + ADR given simultaneously as single doses in BDF_1 mice, Days 1, 5, and 9.

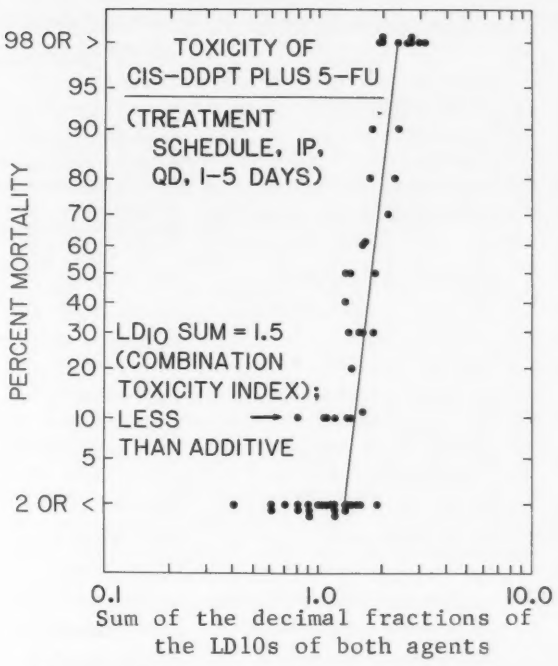

FIGURE 2.—Combination toxicity index of *cis*-platinum + 5-FU given simultaneously as single doses in BDF_1 mice, qd, 1–5 days.

authors' analyses and interpretations of their data in table 2.

We believe that reliable comparisons of the anticancer activity of single drugs and drug combinations require reproducible results which allow quantitative determination of the selective cytotoxicity of drug therapy for tumor cells over vital normal cells. Most of the published experimental combination chemotherapy trials with *cis*-platinum have been done with L1210 leukemia (table 2).

We have described quantitative procedures for reliably estimating the number of viable L1210 and P388 leukemia cells surviving drug therapy (18), and we have used these procedures to quantitate the therapeutic synergism that we have observed when we used *cis*-platinum in combination with a variety of other anticancer agents against these mouse leukemias. Selected examples of the most active two-drug combinations that we have seen are shown in figures 3–6.

cis-Platinum Plus 5-Fluorouracil Against L1210 Leukemia

Figure 3 contains cumulative mortality plots of groups of mice bearing L1210 leukemia treated with *cis*-platinum alone, 5-FU alone, or *cis*-platinum plus 5-FU. We have plotted the optimal therapeutic responses at ≤ LD10 doses from an internally controlled experiment in which tumor-bearing mice were treated with varying doses of the individual drugs and the drug combination. The number of viable tumor cells surviving therapy in each treatment group was estimated from a plot of the lifespan of untreated tumor control mice implanted with a $\log_{10}$ dilution titration ranging from 10^7 to 10^0 counted L1210 cells (18). The data in figure 3 indicate rather marked therapeutic synergism between *cis*-platinum and 5-FU since 60% of the animals treated with the drug combination were cured and the median lifespan (MLS) of the drug-treated mice dying of leukemia exceeded the MLS of those treated with either *cis*-platinum or 5-FU alone.

Figures 4–6 show similar data from combination chemotherapy trials with *cis*-platinum plus ADR (fig 4), *cis*-platinum plus CPA (fig 5), and *cis*-platinum plus VP-16-213 (fig 6) in P388 leukemia.

cis-Platinum Plus Adriamycin Against P388 Leukemia

Data from a similar experiment, in which mice bearing P388 leukemia were treated with *cis*-platinum, ADR, or *cis*-platinum plus ADR, are shown in figure 4. Marked therapeutic synergism was observed. Initiation of treatment was delayed until 5 days after implant of 10^6 P388

TABLE 2.—*cis*-Platinum used in combination with other chemotherapeutic agents

Agents	Tumor system	Comment	Ref No.
2-drug combinations			
cis-Platinum + CPA	Sarcoma 180	Additive, if not synergistic	3
	L1210 leukemia	Synergistic	4–7
	Reticulum cell sarcoma	Synergistic	8, 9
	FANFT-induced bladder carcinoma	Synergistic	10, 11
	Dimethylbenzanthracene-induced mammary tumor	Synergistic	12
cis-Platinum + ifosfamide	L1210 leukemia	Synergistic	7
cis-Platinum + phosphoramide mustard	L1210 leukemia	Synergistic	7
cis-Platinum + Yoshi 864	L1210 leukemia	Synergistic	4, 5
cis-Platinum + BIC	L1210 leukemia	Synergistic	7
cis-Platinum + DTIC	L1210 leukemia	Synergistic	7
cis-Platinum + cytosine arabinoside	L1210 leukemia	Synergistic	4, 5, 7
cis-Platinum + hydroxyurea	L1210 leukemia	Slightly more than additive	4, 5
cis-Platinum + 5-FU	L1210 leukemia	Synergistic	4–7, 13
cis-Platinum + 5-azacytidine	L1210 leukemia	Synergistic	7
cis-Platinum + 6-thioguanine	L1210 leukemia	Synergistic	6
cis-Platinum + methotrexate	L1210 leukemia	Synergistic	4, 6–8
cis-Platinum + bleomycin	B16 melanoma*	Synergistic	16
cis-Platinum + ADR	Sarcoma 180	Synergistic	8
cis-Platinum + vincristine	L1210 leukemia	Synergistic	6
cis-Platinum + vinblastine	L1210 leukemia	Synergistic	6
cis-Platinum + emetine	L1210 leukemia	Synergistic	7
cis-Platinum + camptothecin	L1210 leukemia	Synergistic	7
cis-Platinum + ICRF-159	L1210 leukemia	Synergistic	7, 14, 15
cis-Platinum + SHP†	L1210 leukemia	Synergistic	17
3-drug combinations			
cis-Platinum + CPA + ADR	L1210 leukemia	Synergistic	4
cis-Platinum + CPA + cytosine arabinoside	L1210 leukemia	Synergistic	4, 5
cis-Platinum + CPA + 5-FU	L1210 leukemia	Synergistic	4, 5
cis-Platinum + CPA + hydroxyurea	L1210 leukemia	Synergistic	4, 5
cis-Platinum + CPA + methotrexate	L1210 leukemia	Synergistic	4, 5
cis-Platinum + CPA + Yoshi 864	L1210 leukemia	Synergistic	4, 5

*Active against sc tumor model only.
†Platinum, (1,2-cyclohexanediamine-*N*,*N*')[sulfato(2-)-*O*,*O*']-.

TABLE 3.—Sensitivity and cross-resistance of L1210/0 and L1210/*cis*-platinum (10^5–10^6 cell implant, ip) to single-dose, ip treatment (≤ LD10) with selected alkylating agents

Agent	Log reduction in tumor cells at end of treatment*	
	L1210/0	L1210/*cis*-platinum
cis-Platinum	4	0
BCNU	> 6	0
CCNU	> 6	0
Methyl-CCNU	> 6	1
Chlorozotocin	> 6	1
BIC	6	1
CPA	6	5
Melphalan	6	5
Dianhydrogalactitol	5	4
Piperazinedione	5	5
ThioTEPA	3–4	2

*Unconfirmed except for treatment with *cis*-platinum.

cells, at which time the body burden of viable leukemic cells was about 10^8/mouse (18). Neither *cis*-platinum nor ADR significantly reduced the body burden of viable tumor cells during the treatment period, but the two drugs in combination reduced the body burden by about 3 logs during treatment. We consider this to be rather marked therapeutic synergism against advanced disease.

cis-Platinum Plus Cyclophosphamide Against P388 Leukemia

Figure 5 shows similar data from an experiment in which mice bearing advanced P388 leukemia were treated with *cis*-platinum, CPA, or *cis*-platinum plus CPA. Treatment was delayed until Day 7 after implant of 10^6 P388 cells, at which time the body burden of viable P388 cells was probably > 10^8/mouse (18). CPA alone reduced the body burden of tumor cells by about 5 logs, *cis*-platinum alone reduced the body burden of tumor cells by < 1 log, but the two drugs in combination reduced the body burden by about 8 logs indicating very marked therapeutic synergism. Therapeutic synergism of *cis*-platinum plus CPA has been previously reported for L1210 (4–7), but the degree of increased tumor cell-kill reported for L1210 was far less than can be obtained with P388. In the experiment reported in figure 5, the disease was very advanced at the start of treatment, and the combination of *cis*-platinum plus CPA reduced the viable tumor cell population to a level where a logical prediction of long-term cures should accompany only slight improvement in therapeutic activity.

cis-Platinum Plus VP-16-213 Against P388 Leukemia

Figure 6 shows similar results following treatment of advanced P388 leukemia with *cis*-platinum, VP-16-213,

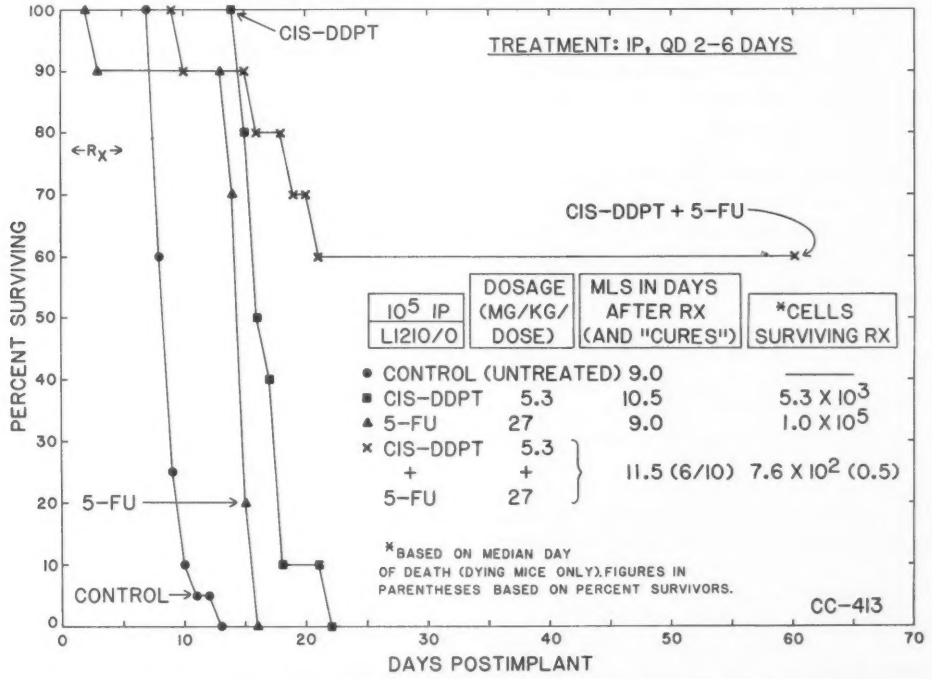
CIS-DDPT
TREATMENT: IP, QD 2–6 DAYS
←Rx→
CIS-DDPT + 5-FU
5-FU
CONTROL→
PERCENT SURVIVING
DAYS POSTIMPLANT
10^5 IP L1210/0
DOSAGE (MG/KG/ DOSE)
MLS IN DAYS AFTER RX (AND "CURES")
*CELLS SURVIVING RX
CONTROL (UNTREATED) 9.0
CIS-DDPT 5.3 10.5 5.3 X 10^3
5-FU 27 9.0 1.0 X 10^5
CIS-DDPT + 5-FU 5.3 + 27 11.5 (6/10) 7.6 X 10^2 (0.5)
*BASED ON MEDIAN DAY OF DEATH (DYING MICE ONLY). FIGURES IN PARENTHESES BASED ON PERCENT SURVIVORS.
CC-413

FIGURE 3.—Therapeutic synergism of *cis*-platinum + 5-FU against L1210/0 leukemia in BDF_1 mice. Cumulative mortality plots of optimal therapeutic responses at ≤ LD10 doses, qd, 2–6 days.

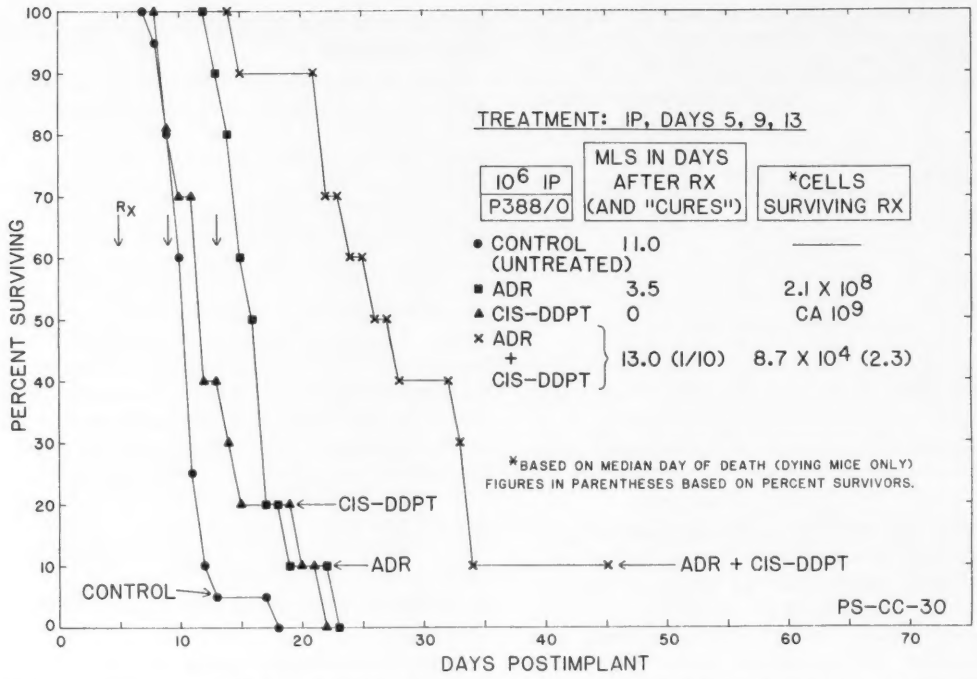
TREATMENT: IP, DAYS 5, 9, 13
10^6 IP P388/0
MLS IN DAYS AFTER RX (AND "CURES")
*CELLS SURVIVING RX
• CONTROL (UNTREATED) 11.0
■ ADR 3.5 2.1 X 10^8
▲ CIS-DDPT 0 CA 10^9
× ADR + CIS-DDPT 13.0 (1/10) 8.7 X 10^4 (2.3)
*BASED ON MEDIAN DAY OF DEATH (DYING MICE ONLY)
FIGURES IN PARENTHESES BASED ON PERCENT SURVIVORS.
Rx
CIS-DDPT
ADR
ADR + CIS-DDPT
CONTROL
PS-CC-30
PERCENT SURVIVING
DAYS POSTIMPLANT

FIGURE 4.—Therapeutic synergism of *cis*-platinum + ADR against advanced P388/0 leukemia in BDF_1 mice. Cumulative mortality plots of optimal therapeutic responses at ≤ LD10 doses, Days 5, 9, and 13.

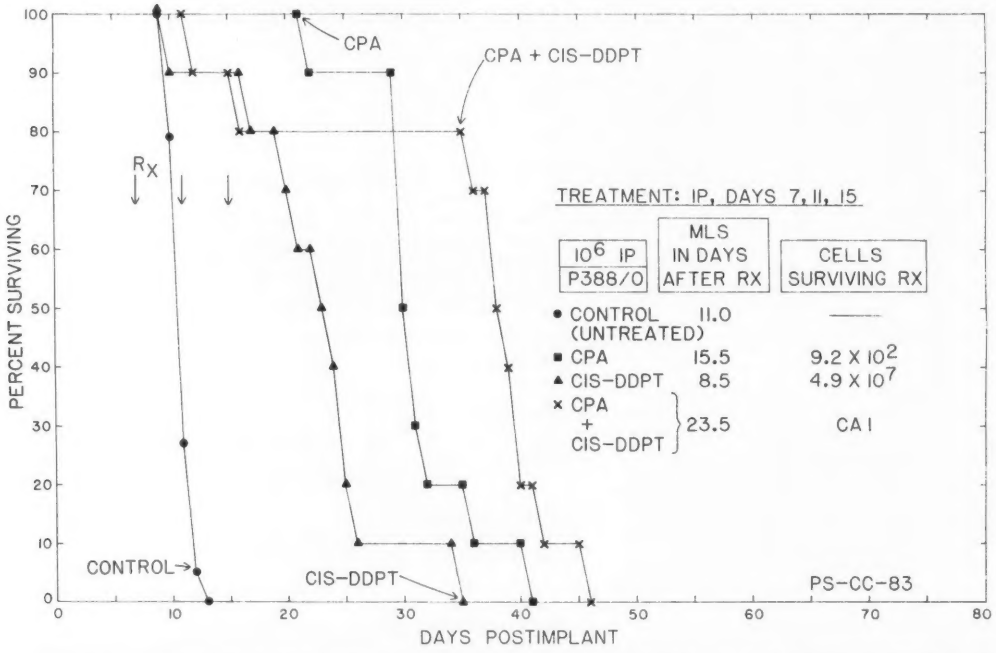

FIGURE 5.—Therapeutic synergism of *cis*-platinum + CPA against advanced P388/0 leukemia in BDF_1 mice. Cumulative mortality plots of optimal therapeutic responses at ≤ LD10 doses, Days 7, 11, and 15.

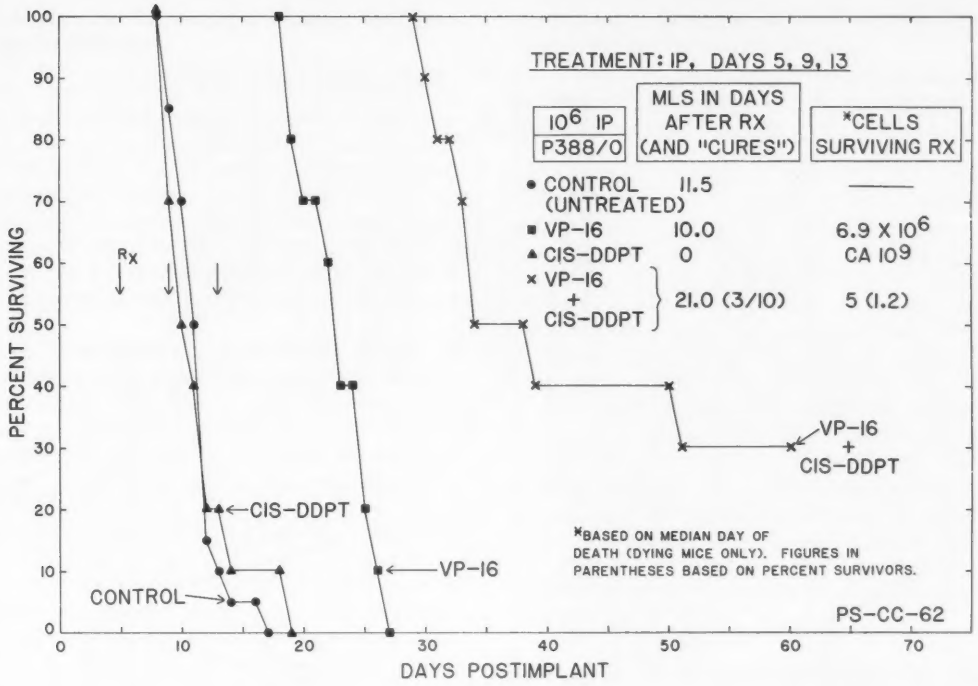
TREATMENT: IP, DAYS 5, 9, 13
10^6 IP P388/0
MLS IN DAYS AFTER RX (AND "CURES")
*CELLS SURVIVING RX
• CONTROL (UNTREATED) 11.5 ——
■ VP-16 10.0 6.9 X 10^6
▲ CIS-DDPT 0 CA 10^9
× VP-16 + CIS-DDPT 21.0 (3/10) 5 (1.2)
Rx
PERCENT SURVIVING
DAYS POSTIMPLANT
CONTROL
CIS-DDPT
VP-16
VP-16 + CIS-DDPT
*BASED ON MEDIAN DAY OF DEATH (DYING MICE ONLY). FIGURES IN PARENTHESES BASED ON PERCENT SURVIVORS.
PS-CC-62

FIGURE 6.—Therapeutic synergism of *cis*-platinum + VP-16-213 against advanced P388/0 leukemia in BDF_1 mice. Cumulative mortality plots of optimal therapeutic responses at ≤ LD10 doses, Days 5, 9, and 13.

or *cis*-platinum plus VP-16-213. Treatment was started on Day 5 after implant of 10^6 P388 cells at which time the body burden of viable tumor cells was about 10^8/mouse. In this experiment, *cis*-platinum alone was ineffective against the advanced disease, VP-16-213 alone reduced the body burden of tumor cells by about one order of magnitude, but the two drugs in combination showed very marked therapeutic synergism.

Estimates of the number of viable tumor cells surviving therapy with *cis*-platinum plus VP-16-213, based on both the MLS of dying mice and long-term cures, indicate that a body burden of about 10^8 tumor cells was reduced to less than ten cells/mouse by drug treatment. Therapeutic synergism of the magnitude shown by *cis*-platinum plus either CPA or VP-16-213 against advanced P388 has not, as far as we know, been seen with any drug combination against any other experimental tumor system. It may exceed that obtainable in the "real world" of cancer chemotherapy in man, but that it was seen against large body burdens of tumor cells (advanced disease) in mice may be very important.

COMBINATION CHEMOTHERAPY OF SOLID TUMORS OF MICE

The minimal clinical response required by the medical oncologist to report a positive effect of drug treatment of solid tumors in man is a ≥ 50% regression of objectively measurable tumors. Until recently, this was seldom seen in the laboratory. Experimental cancer chemotherapists have reported drug treatment as positive and, therefore, effective by inference if drug activity held tumor growth under treatment to ≤ 50% of untreated controls. Even 100% inhibition of growth would be negative to the medical oncologist since he requires actual regression of ≥ 50% of measurable tumors for objective activity by drug treatment. As a result, what the laboratory investigator has called an active antitumor drug in experimental animals has often failed when tested against clinically recognized tumors in man. This should be expected since these different end points for activity have been and are being used for anticancer activity in man and animals. We now have available a sizable number of solid tumors of animals with which regression and sometimes cure of advanced and grossly apparent solid tumors by treatment with representatives of the major classes of clinically useful anticancer drugs can be obtained (19,20).

We have treated a number of solid tumors of mice with *cis*-platinum in combination with other anticancer drugs, in some cases starting treatment when the body burden of tumor cells was very large. Selected examples of the more active drug combinations that we have seen are shown in figures 7–9.

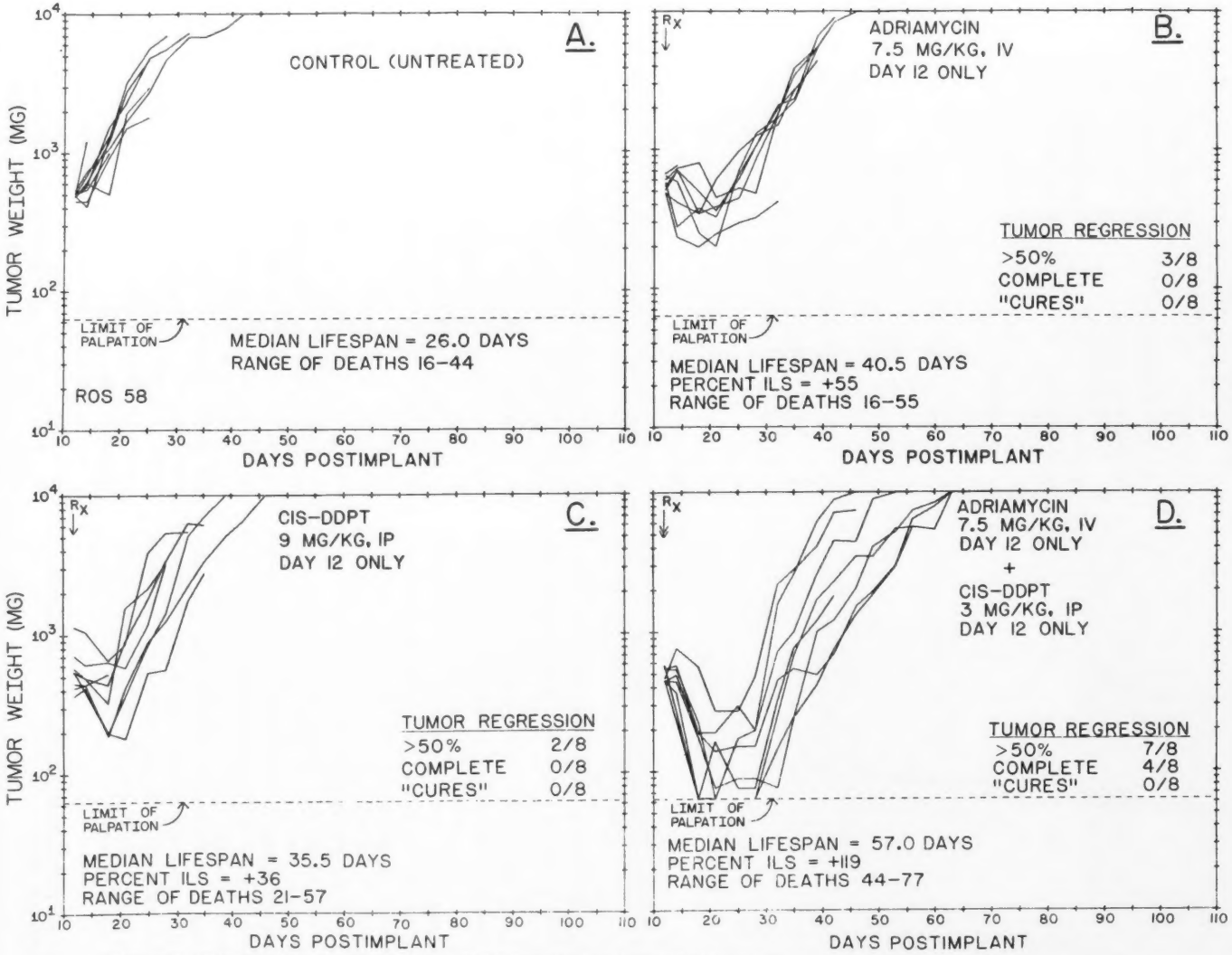

FIGURE 7.—Therapeutic synergism of ADR + *cis*-platinum against advanced Ridgway osteogenic sarcoma. Individual tumor responses of advanced Ridgway osteogenic sarcoma in AKD2F$_1$ mice to single doses of *cis*-platinum, ADR, or *cis*-platinum + ADR. Maximum response to ADR (plot B), *cis*-platinum (plot C), or *cis*-platinum + ADR (plot D). Tumor responses plotted were optimal from a dose-response study containing doses ranging from obviously lethal doses to inactive doses of each drug treatment.

cis-Platinum Plus Adriamycin Against Ridgway Osteogenic Sarcoma

Figures 7 and 8 show the response of individual Ridgway osteogenic sarcomas to treatment with *cis*-platinum, ADR, or *cis*-platinum plus ADR. Data plotted in figures 7 and 8 are optimal drug responses taken at equitoxic doses from a dose-response study of each treatment (*cis*-platinum or ADR used alone and *cis*-platinum plus ADR). All data were obtained from a single internally controlled experiment which is an absolute requirement for reliable activity comparisons with this type of chemotherapeutic analysis.

Single-dose treatment was given on Day 12 after implant when the tumors under treatment ranged from 350 to 1200 mg in size. Limited partial regression rates (20%–30%) were seen with both *cis*-platinum and ADR when used alone, but treatment with *cis*-platinum plus ADR in combination resulted in an 87% partial regression rate, a 50% complete regression rate, and a very marked increase in lifespan (ILS) over that seen with either drug alone.

Figure 8 shows similar data from an experiment in which ADR was given as a single dose on Day 12 after implant and *cis*-platinum was given q4days × 3 starting on Day 12. In this experiment, the single-dose LD10 of ADR and the total-dose LD10 of *cis*-platinum were higher than in the previous experiment (fig 7). Under the treatment doses and schedules used in figure 8, higher regression rates with all drug treatments were obtained and no clear therapeutic synergism of *cis*-platinum plus ADR was seen, pointing out, as is commonly seen in such experimental studies, the marked dependence on both drug dose and treatment schedule for optimal therapeutic responses.

cis-Platinum Plus Cyclophosphamide Against Ridgway Osteogenic Sarcoma

cis-Platinum plus CPA, therapeutically synergistic against L1210 (4–7) and advanced P388 (fig 5), shows marked therapeutic synergism against advanced Ridgway osteogenic sarcoma (fig 9). Drug treatment was started on Day 18 after implant when the individual tumors ranged from 600 to 5000 mg. Repeated courses of sequential drug treatment were used: *cis*-platinum, two doses 3 days apart, q14days × 4; CPA, q14days × 4; and the combination, same schedule except that the CPA was given 24 hours after the first dose of *cis*-platinum in each course of treatment. Both *cis*-platinum and CPA treatment, when used alone, resulted in a 100% partial regression and a 60%–70% complete regression rate, but only *cis*-platinum treatment obtained cures (20%). *cis*-Platinum plus CPA, however, yielded 100% complete regressions, a 40% cure rate, and a very marked ILS of dying animals. (Cured animals were *not* included in calculating the ILS of treated animals.)

cis-Platinum Plus Carminomycin Against Colon Tumor 26

Figure 10 is a cumulative mortality plot of mice implanted ip with a 1.0% tumor brei implant and treated on Day 3 after implant (early treatment of grossly inapparent tumor) with *cis*-platinum, carminomycin, or the two-drug combination. The data plotted are optimal responses at ≤ LD10 doses from internally controlled dose-response groups from each drug treatment. The 0.1% tumor brei control group was included to aid in quantitating tumor cell-kill by drug treatment. Carminomycin alone resulted in about a 1-log cell-kill, *cis*-platinum alone resulted in perhaps a slightly higher cell-kill, but the two-drug combination was therapeutically synergistic because the net tumor cell-kill probably approached an additional order of magnitude compared to either drug alone. This is consistent with the reported therapeutic synergism of *cis*-platinum plus ADR against sarcoma 180 (8), P388 leukemia (fig 4), and Ridgway osteogenic sarcoma (fig 7).

cis-Platinum, a Chemotherapeutic Adjuvant to Surgery

Figure 11 shows data from two experiments in which advanced colon tumor 26 (400–1000-mg tumor size) was treated with surgical adjuvant chemotherapy with *cis*-platinum, CPA, or *cis*-platinum plus CPA following surgical removal of the primary tumor. More than 60% of mice bearing colon tumor 26 of this size have established, and ultimately fatal, metastases (21). Neither *cis*-platinum, CPA, nor *cis*-platinum plus CPA showed any significant activity against the total body burden of tumor cells (including the primary). No significant increase in long-term cures over that obtained by surgery alone was seen with surgical adjuvant chemotherapy, but surgery followed by treatment with either *cis*-platinum alone or *cis*-platinum plus CPA markedly extended the lifespan of animals dying of metastatic disease. (All surgically treated animals that died showed extensive metastatic disease.)

CROSS-RESISTANCE STUDIES WITH *cis*-PLATINUM

The third asset needed by a drug combination is lack of cross-resistance of tumor cells resistant to one or more drugs in the combination to other drugs in the combination. Knowledge about resistance and cross-resistance is important in drug selection for combination drug treatment, and it has the added advantage of providing indications for likely biologic and biochemical mechanisms

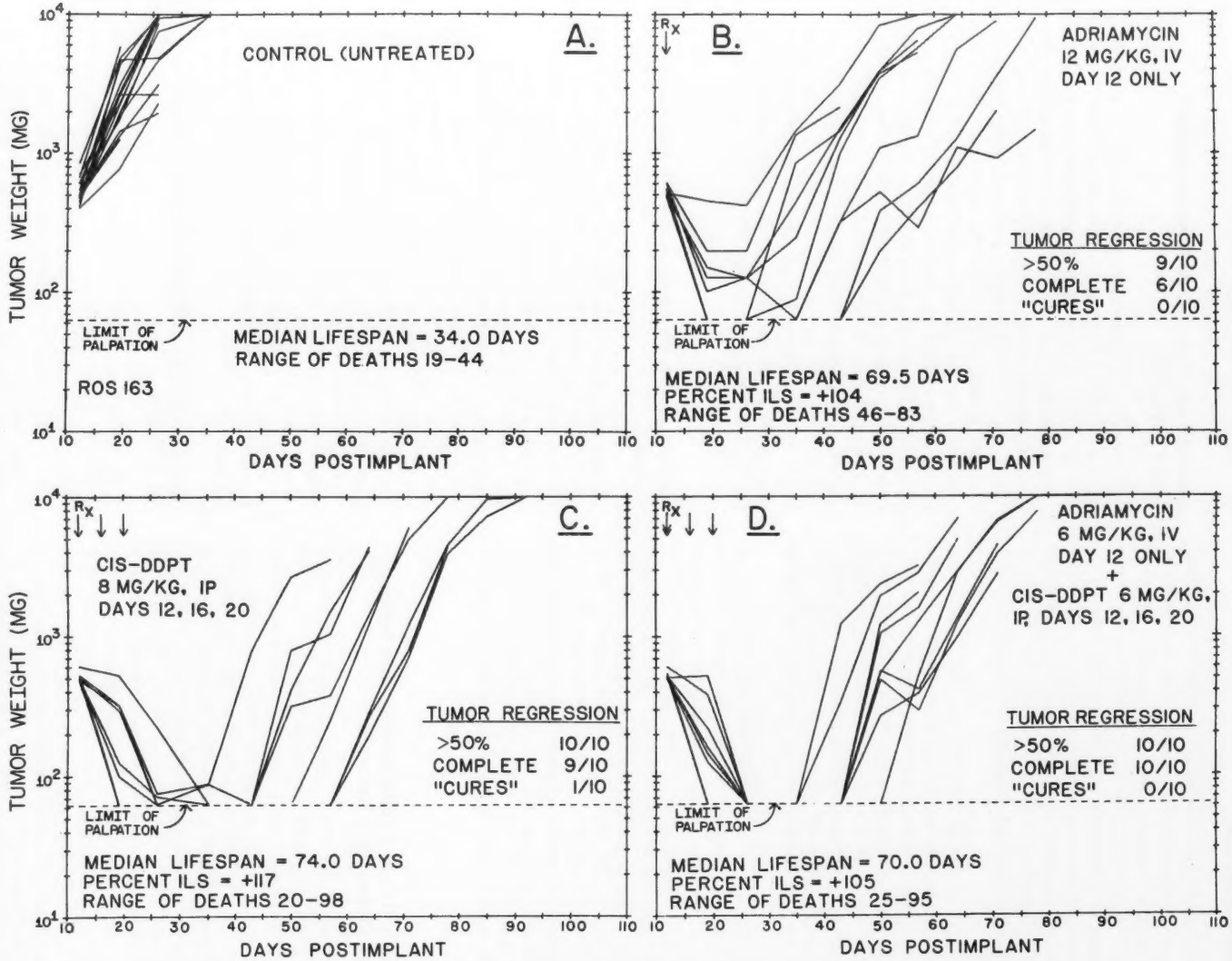

FIGURE 8.—Individual tumor responses of advanced Ridgway osteogenic sarcoma in $AKD2F_1$ mice treated with ADR on Day 12 after implant (plot B); *cis*-platinum on Days 12, 16, and 20 after implant (plot C); or ADR, Day 12 only, plus *cis*-platinum, Days 12, 16, and 20 (plot D). Tumor responses shown were optimal at ≤ LD10 doses from a dose-response study ranging from obviously lethal doses to inactive doses of each drug treatment.

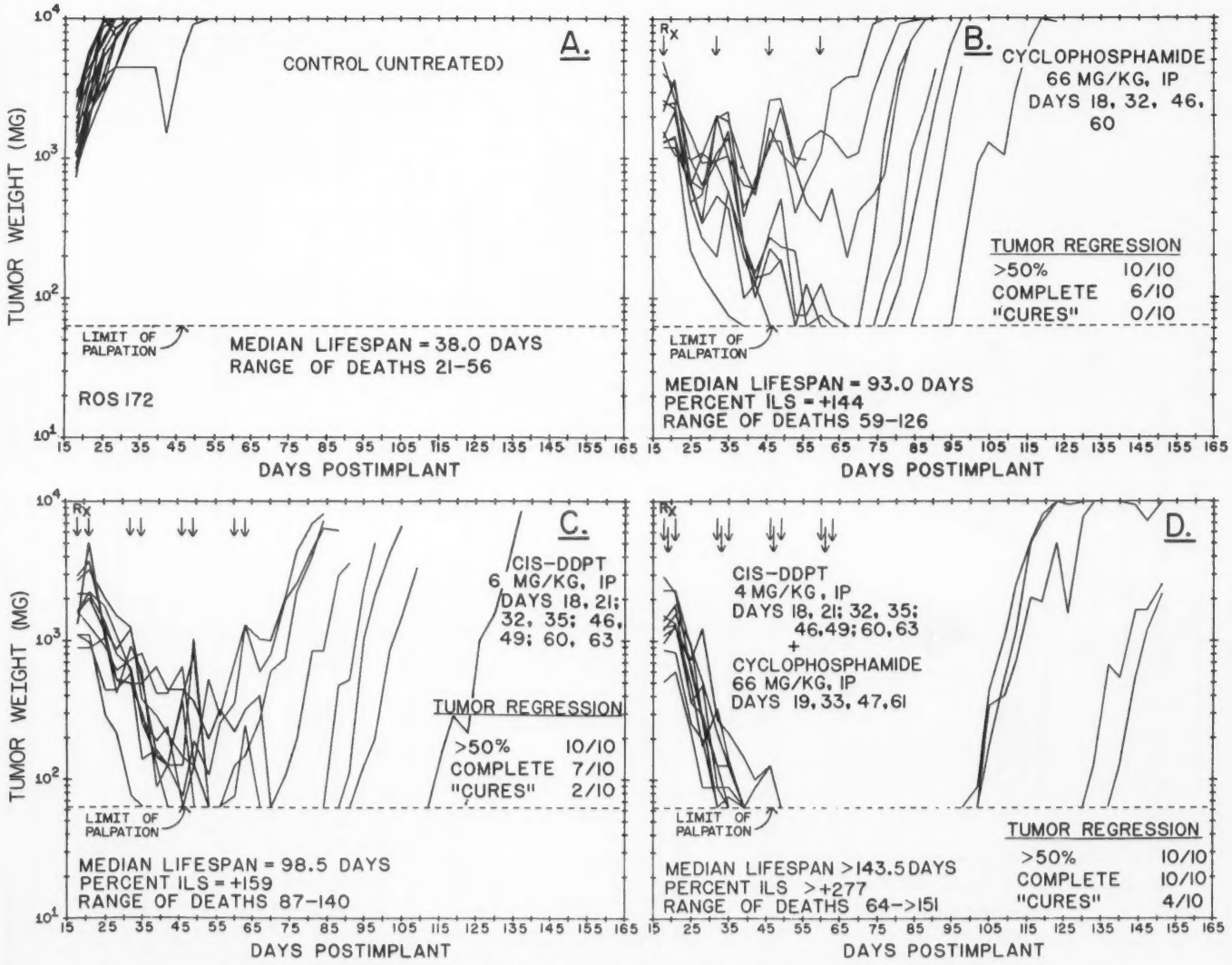

FIGURE 9.—Therapeutic synergism of CPA + *cis*-platinum against advanced Ridgway osteogenic sarcoma. Individual tumor responses of advanced Ridgway osteogenic sarcoma in AKD2F$_1$ mice treated with CPA, Days 18, 32, 46, and 60 after implant (plot B); *cis*-platinum, Days 18, 21, 32, 35, 46, 49, 60, and 63 after implant (plot C); and *cis*-platinum, Days 18, 21, 32, 35, 46, 49, 60, and 63, plus CPA, Days 19, 33, 47, and 61 after implant (plot D). Tumor responses shown were optimal at ≤ LD10 doses from a dose-response study ranging from obviously lethal doses to inactive doses of each drug treatment.

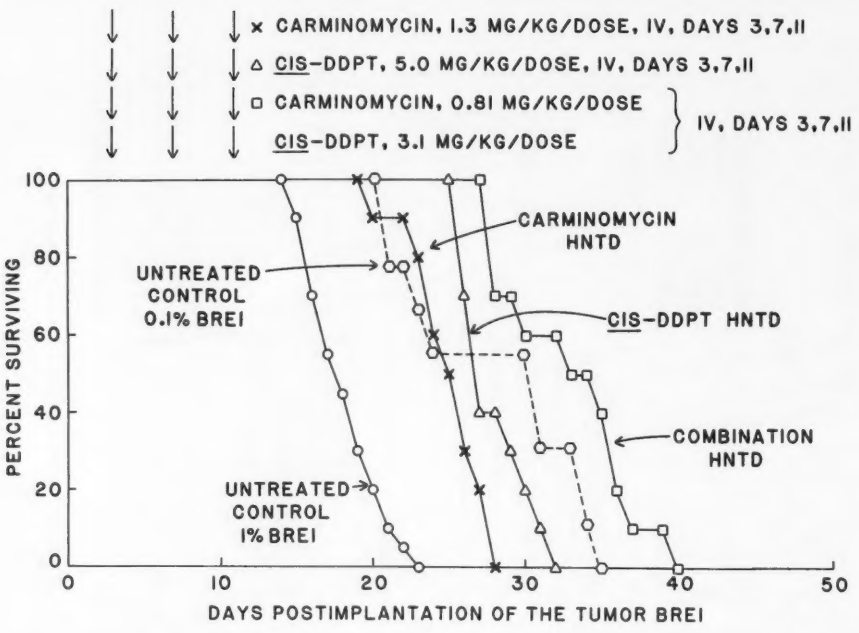

FIGURE 10.—Therapeutic synergism of *cis*-platinum + carminomycin against ip implanted colon tumor 26 in BALB/c mice. Cumulative mortality plots of groups of mice treated with ≤ LD10 doses of carminomycin, *cis*-platinum, or carminomycin + *cis*-platinum. Tumor responses shown were optimal from dose-response groups treated with doses ranging from lethal to inactive doses of each drug treatment.

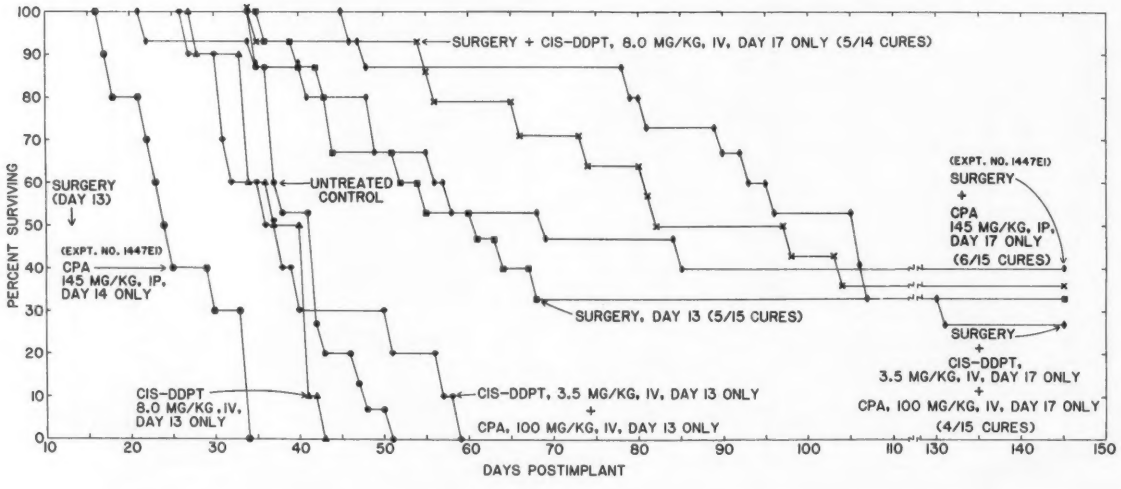
SURGERY + CIS-DDPT, 8.0 MG/KG, IV, DAY 17 ONLY (5/14 CURES)
SURGERY (DAY 13)
UNTREATED CONTROL
(EXPT. NO. 1447EI)
SURGERY + CPA 145 MG/KG, IP, DAY 17 ONLY (6/15 CURES)
(EXPT. NO. 1447EI) CPA 145 MG/KG, IP, DAY 14 ONLY
SURGERY, DAY 13 (5/15 CURES)
SURGERY + CIS-DDPT, 3.5 MG/KG, IV, DAY 17 ONLY + CPA, 100 MG/KG, IV, DAY 17 ONLY (4/15 CURES)
CIS-DDPT 8.0 MG/KG, IV, DAY 13 ONLY
CIS-DDPT, 3.5 MG/KG, IV, DAY 13 ONLY + CPA, 100 MG/KG, IV, DAY 13 ONLY
PERCENT SURVIVING
DAYS POSTIMPLANT

FIGURE 11.—Surgical adjuvant chemotherapy of colon tumor 26 in BALB/c mice. Cumulative mortality plots in groups of mice treated with chemotherapy only, surgery only, or surgery followed by CPA or *cis*-platinum alone or surgery followed by CPA + *cis*-platinum. Treatment was administered when tumors were 400–1000 mg in size.

TABLE 4.—Sensitivity and cross-resistance of L1210 and P388 selected for resistance to selected alkylating agents or *cis*-platinum to optimal single-dose treatment (≤ LD10)

Agent	Log reduction in tumor cells at end of treatment				
	L1210/0	*L1210/CPA*	*L1210/ melphalan*	*L1210/BCNU*	*L1210/ cis-platinum**
CPA	6	0	4	6	5
Melphalan	6	4	2	6	5
BCNU	> 6	> 6	6	2	0
cis-Platinum	4	4†	0	5	0
	P388/0	*P388/CPA*	*P388/ melphalan*	*P388/BCNU*	*P388/ cis-platinum*
CPA	> 6	0–2	7	—	—
Melphalan	> 6	> 6	1	—	—
BCNU	> 6	> 6	7	—	—
cis-Platinum	6	6	0	—	0

*Unconfirmed.
†qd, 1–5 days.

TABLE 5.—Net $\log_{10}$ reduction in viable P388 leukemia cells following optimal treatment of mice implanted ip with 10^6 tumor cells

Agent	Treatment schedule	Net $\log_{10}$ reduction in tumor cells			
		P388/0	P388/ vincristine	P388/ADR	P388/ actinomycin D
Vincristine	q4days × 3	> 6	0	0	0
ADR	Single dose	> 6	4	2	5
Actinomycin D	Single dose	5	3	1	2
cis-Platinum	qd, 1–5	6	4	6	6

of action of new drugs. ". . . testing on tumours with acquired resistance to another class of agent enables the mechanism of action of the new agent to be further evaluated. A cross resistance indicates that somewhere in their mechanism of action the two classes of compound share a common pathway" (22). Initial mechanism of action studies with *cis*-platinum indicated that (*a*) it might be acting bifunctionally to cross-link adjacent nucleophilic centers through covalent binding and thus be reacting like an alkylating agent, or (*b*) it might be acting by binding to or intercalating with DNA (*see* ref 13 for an early limited review of published data up to 1974). Further studies have indicated the more likely possibility that *cis*-platinum may be acting like an alkylating agent (22–24) and may even represent a new functional class of alkylating agents.

We have studied the activity of *cis*-platinum against alkylating agent-resistant sublines of L1210 and P388 and of selected alkylating agents against a line of L1210 selected for resistance to *cis*-platinum (L1210/*cis*-platinum). Some of the results of these studies have been published previously (25). The updated results of these extensive studies are summarized in tables 3–5.

In the work reported here, both the drug-sensitive lines (L1210/0 or P388/0) and the drug-resistant lines (L1210/CPA, L1210/melphalan, L1210/BCNU, L1210/*cis*-platinum, P388/CPA, or P388/melphalan) were included in each cross-resistance trial. Both the drug-sensitive and -resistant tumor sublines were titrated in mice and the log-kill obtained by drug treatment was calculated from the internal tumor control titrations as previously described (18). In addition, except where cited, all of the log-kill data were confirmed in repeated experiments (in some cases, > 20). Thus, the quantitative validity of the data presented within the limits of error of this type of experimental observation has been established.

Burchenal et al (26,27) have isolated sublines of L1210 and P388 resistant to *cis*-platinum which they have kindly given to us. The L1210/*cis*-platinum (26) was used to collect all of the data listed in which we compared the responses of L1210/0 and L1210/*cis*-platinum to *cis*-platinum and other alkylating agents. Table 3 shows the results

of these comparisons. L1210/*cis*-platinum was solidly resistant to *cis*-platinum and showed complete cross-resistance to BCNU and CCNU. It had very limited sensitivity to methyl-CCNU, chlorozotocin, and BIC but retained essentially unchanged sensitivity to CPA, melphalan, dianhydrogalactitol, and piperazinedione. L1210/*cis*-platinum retained somewhat restricted sensitivity to thioTEPA.

The activity of *cis*-platinum against alkylating agent-resistant sublines of L1210 and P388 is shown in table 4. L1210/CPA and L1210/BCNU show no cross-resistance to *cis*-platinum, but L1210/melphalan is completely cross-resistant to *cis*-platinum, as is P388/melphalan. Like L1210/CPA, P388/CPA retains full sensitivity to *cis*-platinum. Connors (22) has also reported that a melphalan-resistant subline of Walker 256 carcinosarcoma (originally very sensitive to melphalan but developing marked resistance to melphalan under drug treatment) was completely cross-resistant to treatment with *cis*-platinum. Of great interest are the unidirectional drug-resistance patterns seen. For example, L1210/melphalan is completely cross-resistant to *cis*-platinum, but L1210/*cis*-platinum retains full sensitivity to melphalan. Similarly, L1210/*cis*-platinum is completely cross-resistant to BCNU, but L1210/BCNU retains full sensitivity to *cis*-platinum.

Similar confirmed data from comparisons of the response of P388/0 and sublines selected for resistance to vincristine (P388/vincristine), to ADR (P388/ADR), and to actinomycin D (P388/actinomycin D) to treatment with *cis*-platinum are listed in table 5. These tumor sublines, selected for resistance to nonalkylating anticancer agents, retain marked sensitivity to *cis*-platinum. P388/ADR and P388/actinomycin D are as sensitive to *cis*-platinum as is the parent drug-sensitive line (P388/0), while P388/vincristine shows limited cross-resistance to *cis*-platinum.

DISCUSSION

The data presented herein support the conclusion, drawn on the basis of biochemical and molecular biologic data (22–24), that *cis*-platinum may be acting as an alkylating agent in demonstrating carcinostatic or cytotoxic activity against drug-sensitive tumor cells in vivo. These data also suggest that *cis*-platinum, if acting like an alkylating agent, may represent still another functional class of alkylating agents, of which at least seven others have been previously suggested or recognized on chemical and/or biologic grounds (25). If this is true, then the wide range of therapeutic synergisms observed (table 2) and the marked degree of increased activity seen with *cis*-platinum in combination with other anticancer drugs against advanced-staged leukemias and solid tumors in animals (figs 3–7 and 9) are consistent with similar activity observed with other alkylating agents in combination with antimetabolites, DNA binders, and especially other alkylating agents (13,19,20,25,28). If the biologic mechanism of action of *cis*-platinum in inhibiting tumor growth and/or killing drug-sensitive tumor cells is "alkylating agent like," then the therapeutic synergisms reported here between *cis*-platinum plus CPA against P388, Ridgway osteogenic sarcoma, and colon tumor 26 further extend this broadly based observation of therapeutic synergism by alkylating agent drug combinations in experimental cancer chemotherapy, and further support the indication for carefully planned clinical trials.

The resistance and cross-resistance data presented support the conclusion that alkylating agents are not all functionally identical or similar. Indeed, very major and possibly practically useful differences exist which can be broadly demonstrated in a variety of drug-sensitive and -resistant tumor systems. This area of developmental cancer chemotherapy has not been seriously pursued by either laboratory or clinical oncologists. Perhaps the entrance of *cis*-platinum into this area of alkylating agent chemotherapy will stimulate further critical and possibly useful work.

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Platinate Toxicity: Past, Present, and Prospects [1]

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SUMMARY

Using traditional toxicologic methods, four species were studied for their qualitative and quantitative predictiveness of the toxic effects of *cis*-dichlorodiammineplatinum(II) in man. Of the four species studied, mouse, monkey, rat, and dog, the latter two gave the best overall results. Using an in vivo rat model, it was found that except for chloroplatinic acid, eight of the tested analogs were less nephrotoxic than the parent drug, *cis*-dichlorodiammineplatinum(II). The in vitro renal toxicity screen using flounder tubules showed that of the 26 compounds studied, about half were less toxic than the parent compound. This in vitro mini-tox system can be performed about 30 times faster and at one fiftieth the cost of the in vivo model. The in vitro studies also provided evidence that the biochemical site of toxicity of platinates is on ATPases. The latter studies suggested a basis for unifying the mechanistic interpretation of the toxic actions on such disparate target organs as the kidney, nerve, stomach, and inner ear.

[Cancer Treat Rep 63:1475–1483, 1979]

This survey is divided into three areas. First, it will provide a review of the traditional toxicologic studies in animals from the point of view of the qualitative and quantitative correlations of effects seen in man. Second, a summary is included of the results of a ranking system applied to rats, with emphasis on the kidney as a target organ of toxicity. This in vivo mini-tox system was developed by the Toxicology Program Office of Battelle Memorial Institute. In the third portion of this report, recent information is provided on a new in vitro, rapid, inexpensive target-organ screen, This latter system offers a method for powerful discrimination and ranking of the relative nephrotoxicity of large numbers of platinate analogs.

MATERIALS AND METHODS

Protocol Toxicity

cis-Dichlorodiammineplatinum(II) (*cis*-platinum) was subjected to toxicologic evaluation, by the iv route, in beagle dogs and rhesus monkeys according to the published protocols (1) of the Laboratory of Toxicology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute. Audiometry studies were done in male and female guinea pigs. The disappearance of Pryer's reflex in response to tones from a Galton whistle (2) served as the indicator for hearing loss. Surface preparations from the organ of Corti were prepared for histopathologic evaluation according to the technique of Johnson and Hawkins (3). The degree of cell

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damage in the organ of Corti was mapped on cochleograms as proposed by Engstrom et al (4).

In Vivo Mini-Tox Model in Rats

The general procedures used in these studies were as described by Ward et al (5). F344 male rats, 6 weeks old and 100–120 g, were used. They were quarantined for approximately 2 weeks prior to the test. Drugs were supplied by the Division of Cancer Treatment, National Cancer Institute. The compounds were formulated for administration as follows. Chloroplatinic acid and *cis*-platinum were administered in normal saline at concentrations of 10 and 0.5 mg/ml respectively. Five percent dextrose in water was used to formulate a 10-mg/ml solution of NSC-241240, a 1-mg/ml solution of NSC-250427, a 20-mg/ml solution of NSC-256927, and 2-mg/ml solutions of NSC-263158 and NSC-268252. A 10-mg/ml solution of NSC-271674 in 2% $NaHCO_3$ was used.

A single-dose iv LD50 experiment was performed for each compound, using a minimum of ten rats per dose and a minimum of five doses. Rats were held for a 14-day observation period following injection. The results from this experiment allowed the LD90, LD50, and LD10 to be estimated by probit analysis.

The following blood parameters were measured in selected animals: hematocrit, wbc and differential leukocyte counts, BUN, creatinine, and SGPT. Necropsies were performed on all rats immediately after death or following terminal bleeding, and all gross lesions were recorded. Organ weights for the right and left kidneys, liver, spleen, and heart were determined. The right kidney (transverse section), left kidney (longitudinal section), liver, lung, duodenum, heart, spleen, pancreas, brain, ileum, colon, cecum, thymus, and sternum were processed for histopathologic examination. A bone marrow smear also was made for M:E ratios.

In order to test the hypothesis that there is a threshold of urinary platinum concentration before manifestation of renal toxicity, male Sprague-Dawley rats (weighing about 150 g) were treated iv with 10 or 8 mg/kg of *cis*-platinum. Half of the treated groups were hydrated by injecting ip a volume of normal saline (0.9%) equal to 25% of the body weight. Rats were hydrated under light ether anesthesia 12 hours and 1 hour prior to *cis*-platinum administration and 8 and 24 hours after drug treatment. Urine was collected periodically for determination of platinum using flameless atomic absorption spectroscopy. Five days after *cis*-platinum administration rats were lightly anesthetized as above, axial blood was collected, and kidney, liver, and duodenum were collected, sectioned, and fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin for histopathologic evaluation.

Isolated Flounder Tubule Assays

The basic procedures were those of Forster (6) and Kinter (7). Forster's bicarbonate (140 mM NaCl, 2.5 mM KCl, 1.0 mM $MgCl_2$, 7.5 mM $NaHCO_3$, 0.5 mM NaH_2PO_4, 1.5 mM $CaCl_2$, and pH adjusted to 8.25 with bubbling CO_2) was used as the incubation medium. Sonication (150 watts bath sonicator from Heat Systems Ultrasonics, Inc, Plainview, NY) and tap water heating (60°C) were often required for solvation of platinum compounds. When this was necessary, the media were filtered through 0.45μ millipore filters immediately prior to incubation to remove undissolved particles.

Flounders (*Pseudopleuronectes americanus*) were caught by trawl off the coast of Mt. Desert Island, Me, and were maintained in tanks with circulating bay water. The tubules were incubated at 17°C with gentle shaking in an oxygen atmosphere throughout the preincubation period. This was followed by the addition of sufficient [^{14}C]- and nonradioactive *p*-aminohippuric acid (PAH) to result in a final concentration of 10 μM in the incubation medium. After 45 minutes the PAH incubation was terminated by removing the tubules from the medium and by adding 1 N NaOH. After correcting counts for background and quench, tissue/media ratios,

$$T/M = \frac{(dpm/g\ tissue)}{(dpm/ml\ media)}$$

were calculated.

Na,K-ATPase activity was assayed using a modification of the procedure of Miller and Kinter (8) where the difference between the inorganic phosphate liberated from ATP in the presence and absence of 0.1 mM (flounder) or 1.0 mM (F344 male rat) ouabain was measured.

Data from assays with inhibitors were compared to data from paired controls and I50 values were obtained from a plot of percent inhibition versus log inhibitor concentration. I50 values obtained from different tissue pools varied by no more than 25%.

RESULTS

Traditional Toxicology

Most of these results were published previously (9,10). The major qualitative toxic effects seen in man were well-predicted by all four of the animal species studied (table 1). As has been our experience with most anticancer drugs studied by our protocol (11), the dog provided excellent predictiveness for the toxic effects seen in man. For *cis*-platinum, the rat, except for its inability to vomit, gave not only as good an estimate of human toxicity as did the dog, but on a retrospective study it also showed ototoxicity. The monkey predicted for toxic effects other

TABLE 1.—Predictability of animal species for toxic effects of *cis*-platinum in man

Principal toxic effects in man	Predictability of toxicity in—*			
	Mouse	Rat	Dog	Monkey
Gastrointestinal				
Emesis	–	–	+	+
Anorexia	ND	+	+	+
Renal				
BUN increase	+	+	+	+
Creatinine increase	ND	+	+	–
Polyuria	ND	+	+	+
Tubular necrosis	+	+	+	+
Hematologic				
Wbc decrease	ND	+	+	+
Platelet decrease	ND	+	+	–
Bone marrow hypocellularity	+	+	+	+
Otologic†	ND	+	ND	+

*ND = not determined; + = sign was seen in animals and man; – = sign was seen in man but not in animals.

†Otologic toxicity was also noted in the guinea pig.

TABLE 2.—Quantitative toxicity of *cis*-platinum in man and animals

	Defined iv doses (mg/m^2)							
	Human		Beagle dog		Rhesus monkey	Rat		Mouse
	Single dose	Daily doses × 5	Single dose	Daily doses × 5	Daily doses × 5	Single dose	Daily doses × 5	Single dose
Frequently used clinical schedules	75–100	20						
Lethal dose			100	30	30			
Toxic dose high			50	15	15			
Toxic dose low			25	7.5	3.5			
Highest non-toxic dose			12.5	3.5	1.7			
LD90						49	—	48
LD50						44	13	39
LD10						41	11	33

than increases in plasma creatinine and decreases in the platelet count. While the mouse studies were not pursued as vigorously as the studies in the other species, nonetheless they demonstrated renal and marrow toxicity.

The quantitative toxicity of *cis*-platinum was assessed in four species (table 2). The HNTD (highest non-toxic dose), TDL (toxic dose low), TDH (toxic dose high), and LDs (lethal doses) in dogs and monkeys are as defined in our protocol (1). For both the single-dose and the daily × 5 studies in dogs, there is the classic doubling of the dose which gives the shape of the dose-response curve. In the monkey there were several "missed" dose intervals between the TDH and the TDL. In rodents we use the standard LD10, LD50, and LD90 method to define these doses, and the steepness of the dose-response curve is noteworthy.

In Vivo Toxicity Ranking System

An approach for ranking the relative toxicity of platinum compounds is shown in table 3. This ranking system allows one to get an overall toxicity rating or to focus on an individual toxicologic parameter to compare analogs. All drugs tested to date have generally been less toxic than the parent compound (*see* table 4) (compare values in bottom row of table with values for *cis*-platinum), and except for chloroplatinic acid all drugs tested are specifically less nephrotoxic than the parent compound. Furthermore, since chloroplatinic acid has been negative in rodent tumor systems, the toxic and therapeutic effects in this case are clearly distinguished.

This ranking system is, of course, only as good as are the data put into it. We are particularly uncomfortable

TABLE 3.—Scoring system used for comparative toxicity of platinum compounds

Parameter	Scoring system and definitions
Hematocrit, wbc count	1 = < 20% decrease 2 = 20%–50% decrease 3 = > 50% decrease
BUN, creatinine, SGPT	1 = < 50% increase 2 = 50%–200% increase 3 = > 200% increase
Body weight loss	1 = no weight loss (may be slowing of growth) 2 = < 10% weight loss 3 = > 10% weight loss
Histopathology	1 = no lesions 2 = mild lesions in a few animals 3 = moderate to marked lesions 4 = marked to severe lesions

about problems of variability, reproducibility, and quantification. A number of in vitro systems can be run under conditions difficult to standardize with in vivo systems.

In Vitro Screen: Isolated Tubule Studies

In vitro tests have always appealed to pharmacologists, both as a screening tool and in the study of mechanisms of action of drugs. Such systems become especially powerful if they bear some relationship to toxic parameters in the intact animals and in man (12). A key problem, then, is related to the strength of the foundation built for the in vivo-in vitro bridge. If the response under study is related to the whole-animal response, then the in vitro assay can be regarded confidently as "a fundamental, predictively useful parameter in the expression of animal toxicity" (12). In building one side of the in vivo-in vitro bridge we, of course, began with a clear definition of the major, dose-limiting toxicity observed in man and animals, ie, renal tubular necrosis. Construction on the other side of the bridge actually was started decades ago by National Institutes of Health intramural support of comparative physiology and pharmacology.

Recognizing the potential of this method 30 years ago, Forster (6) said that it "would be useful in rapid screening to test the possible effects of chemicals and physical agents on renal tubular secretion." Thus, 30 years later we put this concept to work in attempting to find a less nephrotoxic agent among the many hundreds of platinum analogs now synthesized by drug development programs around the world.

The time course of inhibition of tubular transport of PAH by *cis*-platinum (table 5) was extremely interesting from several points of view: unlike inhibitors such as organic acids and cyanide (13) which show virtually instantaneous effects, platinum compounds did not show significant inhibition until incubated for 6 hours. Mean values appear in table 5 and the variability of the assay was about 10%. At lower concentrations and at early times there was apparently some stimulation of PAH transport; the phenomenon is not unusual in studying transport or enzyme effects of heavy metals. The degree

TABLE 4.—Comparative toxicity (*see* table 2 for scoring system) of platinum compounds after a single iv injection in male F344 rats

	Compound (NSC No.)							
Parameter	119875 (*cis*-platinum)	4958	241240	250427	256927	263158	268252	271674
Hematocrit	1	1	3	1	2	1	1	1
Wbc count	3	1	2	2	3	1	1	3
BUN	3	3*	1	1	1	1	1	1
Creatinine	3	3*	1	1	1	1	1	1
SGPT	1	1	1	2	1	3	2	1
Body weight loss	3	1	1	2	2	2	2	3
Histopathology						†		
Renal	4	4	1	3	3	3	3	2
Lymphatic	4	2	1	4	4	3	3	1
Hematopoietic	3	1	4	3	3	4	4	4
Gastrointestinal	4	1	1	3	1	3	3	1
Total score	29	18	16	22	21	22	21	18

*Actual levels for BUN and creatinine were 2 times those obtained with *cis*-platinum.
†At the LD50 level 1 animal had marked hepatic necrosis.

TABLE 5.—Time course of inhibition of PAH uptake by *cis*-platinum-treated flounder tubules*

Preincubation time (hrs)	% of control T/M†	
	1.0 mM	5.0 mM
0	91	91
1	133	80
2	106	67
6	24	3
24	30	—

*Experimental details are in the Materials and Methods section.

†*See* text for definition of T/M.

of inhibition at 24 hours was about the same as at 6 hours; furthermore, this is about the upper limit of survival of the isolated tubules in this system. As expected, the higher dose (5 mM) of *cis*-platinum produced inhibition earlier than did the lower dose. Of note, we have found that the diuretic furosemide is potently toxic (I50 = 0.01 mM) to tubules in this system, and that this diuretic enhanced the *cis*-platinum inhibition of PAH uptake by about 50%.

The results of the I50 studies on PAH uptake of 11 analogs appear in table 6. Although not shown here, semilogarithmic plots of concentration versus percent inhibition showed a linear response, thus fulfilling an important requisite of such systems (12). The most potent platinum compound we have tested to date is the highly nephrotoxic chemical chloroplatinic acid (5); its I50 is 10 μM while that of the parent drug is 0.8 mM. Also shown in table 6 are the results of ATPase assays on isolated flounder tubules and homogenates of rat renal cortex. With the exception of NSC-241240 (data not shown; at 0.1 and 1.0 mM there was slight stimulation of ATPase), all platinum compounds tested did inhibit both flounder and rat renal Na,K-ATPase. I50 values ranged from 1 μM to 5 mM, with chloroplatinic acid and NSC-263158 being the most potent. In comparison, I50 values for the specific Na,K-ATPase inhibitor, ouabain, are approximately 1 μM and 0.1 mM for the flounder and rat renal enzymes respectively. In contrast, I50 values for platinum compound inhibition of Ca-ATPase or Mg-ATPase activity in these preparations were at least one to two orders of magnitude higher than those for Na,K-ATPase (data not shown). Significantly, for the four compounds for which we present comparative data, the rank order of relative inhibitory potency is similar, with only the order of two of the strongest inhibitors (which possess similar I50 values in both species) being reversed. Further comparison with data on PAH uptake (table 6) shows a favorable rank order of I50 values, with particularly strong correlation for the most potent platinates. Interestingly, all of the cyclohexanediamine compounds (NSC-224964, NSC-250427, NSC-263158, NSC-268252, and NSC-271674) are quite potent inhibitors of tubule transport of PAH (0.05–0.25 mM), and all of those tested in the ATPase systems are more potent than *cis*-platinum. NSC-256927 and NSC-267583 had the least tubule toxicity in this series.

TABLE 6.—Inhibition of PAH uptake and ATPase by platinum compounds*

NSC No.	Chemical name	PAH uptake: I50 (mM)	ATPase: I50 (mM)	
			Flounder	Rat
4958	Platinate(2-), hexachlorodihydrogen, hexahydrate	0.01	0.001	0.06
119875	Platinum, dichlorodiammine(II)-, *cis*-	0.8	5.0	2.0
131558	Platinum, dichlorodiammine(II)-, *trans*-	0.5	—	—
224964	Platinum, (1,2-cyclohexanediamine-*N*,*N*′)[propanedioato(2-)-*O*,*O*′]-, (*SP*-4-2)-	0.15	1.0	—
247541	Discreet	1.0	—	—
250427	Platinum, (1,2-cyclohexanediamine-*N*,*N*′)[sulfato(2-)-*O*,*O*′]-, (*SP*-4-2)-	0.1	0.2	0.5
256927	Platinum, dichlorodihydroxybis(2-propanamine)- (*OC*-6-33)-	2.5	0.5	—
263158	Platinum, (1,2-cyclohexanediamine-*N*,*N*′)bis(methanesulfonato-*O*)-, (*SP*-4-2)-	0.05	0.005	0.04
267583	Platinum(2+), diamminebis(guanosine-N^7)-, dichloride (*SP*-4-2)-	4.0	—	—
268252	Platinum, (1,2-cyclohexanediamine-*N*,*N*′)dihydroxy-, (*SP*-4-2)-	0.25	—	—
271674	Platinate(1-), [1,2,4-benzenetricarboxylato(3-)-O^1,O^2](1,2-cyclohexanediamine-*N*,*N*′)-, hydrogen, (*SP*-4-3)-	0.2	0.1	—

*Experimental details and methods for calculations are in the Materials and Methods section.

TABLE 7.—In vitro-in vivo correlations of platinates: rank order (higher Nos. mean higher activity/potency)

NSC No.	Flounder tubule assay	Optimal dose* (daily, Days 1–9)
267583	1	1
256927	2	2
119875 (*cis*-platinum)	3	4
268252	4	3
271674	5	5
263158	6	6

*L1210 data from screening files provided by Dr. Mary K. Wolpert.

A major conclusion from this table is that the in vitro tubule assay can very accurately discriminate between a 200–5000-fold range (PAH I50s of 0.01–4 mM or ATPase I50s of 0.001–5 mM) of relative toxicity. Such discrimination is obviously not available from the +1 to +4 type rating system of histopathologic evaluation nor is it available from BUN values since the highest concentration of this parameter we have seen in rats is < 200 mg/100 ml.

We should now consider if this ranking corresponds to any whole-animal data. There are only a few compounds where truly comparative data exist, primarily because so many substances were tested in suspension in the mouse tumor systems. Nonetheless, we obtained L1210 screening data from M. K. Wolpert (Division of Cancer Treatment, National Cancer Institute), and the optimal doses for the six compounds given daily for 9 days were compared (table 7) with the flounder assay. Note that except for a 3,4 reversal, the rankings are the same. Preliminary data were obtained on the effects on tubules of just two concentrations (rather than more through I50 experiments) for 14 additional platinum compounds. Using the information gathered in previous work we estimated that testing compounds at 1.0 and 0.1 mM would give us a rough idea of how nephrotoxic a compound was relative to the parent compound. Since *cis*-platinum produced 75% inhibition at 1 mM, all of those with > 75% inhibition are regarded as more nephrotoxic. In these abbreviated studies, the following compounds were more potent renal toxic agents than *cis*-platinum: NSC-141523, NSC-146067, NSC-153148, NSC-153149, NSC-157981, NSC-170896, NSC-236232, and NSC-239851. The remaining drugs tested were less potent than *cis*-platinum: NSC-146068, NSC-170897, NSC-194814, NSC-241240, NSC-255058, and NSC-263688. While we were unable to solubilize all of the compounds added to the incubation medium for eight of those in the above listing, enough of every compound tested (26 total) *did* dissolve sufficiently to give some inhibition of PAH uptake by tubules. While these values are not corrected for true platinum concentration, they represent total drug added to the incubation medium and therefore would show greater potency (ie, toxicity) when only dissolved drug is considered. Among the compounds which were soluble we would encourage the further development of the following, which are less nephrotoxic than *cis*-platinum even if therapeutically equivalent to the parent compound: NSC-146068, NSC-241240, NSC-247541, NSC-255058, NSC-256927, NSC-267583, and NSC-268252.

Application of Flounder Data to Mammals

The relevance of such studies to the rat was directly tested both in vivo and in vitro. Our three-part hypothesis is that *cis*-platinum is a direct tubule toxicant, that it must be present in urine for a few hours at 1 mM (about 200 μg/ml), and that its biochemical mechanism of toxicity is mediated via inhibition of ATPase. A corollary would be that properly hydrated animals (and presumably humans) treated with *cis*-platinum would show less toxicity than those not vigorously hydrated, ie, *cis*-platinum-treated controls. Using 10 mg/kg of *cis*-platinum, 60% of the drug-alone group were dead within 1 week, while in rats treated with *cis*-platinum plus hydration, there were no deaths in this time period. At 8 mg/kg of *cis*-platinum there were no deaths in any of the treatment groups (table 8), and therefore information on clinical chemistry and hematologic studies are more meaningful. The overall toxicity, as assessed by changes in initial body weight, was not altered by hydration. Note that urinary platinum levels in hydrated animals receiving *cis*-platinum never reached mean values of > 126 μg/ml, while for up to 2 hours the levels exceeded 265 μg/ml in the nonhydrated animals receiving *cis*-platinum. Two important indicators of nephrotoxicity, BUN and creatinine, were elevated in both *cis*-platinum-treated groups, while hydration reduced the severity of these parameters by half. Meanwhile, the effects of *cis*-platinum on wbcs, rbcs, and hemoglobin were not attenuated by the hydration treatment. As in the case of body weight changes, this kind of discrimination between the renal and hematologic target organs is extremely important because it suggests that the therapeutic effect of *cis*-platinum may not be reversed by hydration, while the nephrotoxicity is lessened by these procedures.

DISCUSSION

As has been our general experience, animal studies predicted both the qualitative and quantitative toxicity of *cis*-platinum, including the nephrotoxicity, which is not as commonly predicted in toxicity studies of antineoplastic agents as are gastrointestinal and hematologic toxic effects (11). With the emergence of nearly 1000 platinates (14), the problem has become one of selecting those with less nephrotoxicity from among those with at least the same therapeutic effects as *cis*-platinum. One alternative,

TABLE 8.—Effects of *cis*-platinum (8 mg/kg) and hydration in rats*

	Change in body weight (% in 6 days)	Urine volume (ml/24 hrs)	Urinary platinum (μg/ml): hrs after injection of *cis*-platinum					BUN (mg/100 ml)	Creatinine (mg/100 ml)	Wbcs (× 10^3/mm^3)	Rbcs (× 10^3/mm^3)	Hemoglobin (g/100 ml)
			0.5	1	2	4	24					
Control	+30	5.3 ± 0.9	—	—	—	—	—	21 ± 2	0.6 ± 0.1	13.3 ± 1.1	6.7 ± 0.3	14.5 ± 0.1
Control + hydration	+24	43.8 ± 3.7	—	—	—	—	—	20 ±13	0.6 ± 0.1	13.3 ± 1.9	6.6 ± 0.3	14.5 ± 0.4
cis-Platinum	–25	6.1 ± 3.5	373 ± 28	265 ± 68	66 ± 37	30 ± 10	21 ± 9	75 ± 38	2.6 ± 1.1	6.1 ± 1.8	7.5 ± 0.6	17.0 ± 0.9
cis-Platinum + hydration	–26	44.3 ± 5.9	126 ± 51	91 ± 28	31 ± 5	7 ± 1	1 ± 1	44 ± 23	1.6 ± 0.4	6.5 ± 1.2	8.0 ± 0.2	17.4 ± 0.2

*Values are mean ± SD for 4–5 Sprague-Dawley rats/group.

which never was seriously considered, is that of subjecting a number of these compounds to our large animal protocol (1) to see which are less toxic than the parent drug. This alternative has two problems: cost and criteria for selection. Since the current price of our protocol studies is about $100,000/drug, we could test very few compounds before our budget would be depleted. The more important question is what criteria are to be used to determine which analogs should be tested further. Two initial criteria were adopted: (*a*) obtain antitumor data in rodent systems, and (*b*) when adequate activity is demonstrated, a minimum aqueous solubility limit (1 mg/ml) is set. This latter decision is not without hazard since at this stage of our drug development program, no formulation work has been done and thus a promising compound might be discarded for the lack of these types of studies. On the other hand, our program also lacks the resources to study the formulation problems of 1000 drugs/year. Thus, using a tier-type concept of testing (15), we next tried testing rats as the initial species, with the idea that only the most promising agents would go on to be evaluated in the next tier, ie, large animals. Obviously, the next tier would be a phase I clinical trial. While this rat mini-tox system had a size advantage, requiring less of the usually scarce analog, it still had the built-in restriction concerning drug solubility. Thus, even if all possible routes of injection were used, one still could not administer, for example, 1000 ml of drug solution to a 200-g animal. While the rat model costs about one tenth that of the large animal protocol, it does not save a considerable amount of time because of the time required for clinical chemistry studies, hematologic studies, and, especially, histopathologic processing and evaluation. Nonetheless, this rat model did provide information useful enough to suggest large animal studies for two (NSC-241240 and NSC-271674) of the eight compounds studied to date. Furthermore, this model showed that it was sensitive enough to call attention to the "nearly pure nephrotoxic" potential of chloroplatinic acid (table 4). In spite of the advantages of this system, it simply cannot reasonably serve the time and financial constraints of an analog developmental program as large as the one for platinates. Thus, against these needs and the solubility problems of platinum analogs, we developed the in vitro flounder model. As has been done with other in vitro systems (12), this one can serve to retain good candidate drugs and eliminate the poor ones. As has been shown here, this isolated tubule model of PAH transport provided more advantages than disadvantages when used as the proposed first tier. In the second large animal tier, the importance of pharmacologic factors and other influences found in the intact animal would be uncovered.

On the other hand, the simplified tubule preparation allowed for the direct assessment of the nephrotoxicity of platinum analogs. Thus, the time course experiments (table 5) could not reasonably be done in vivo, and yet they produced information which was immediately "fed back" into the rat studies (table 8) which, in turn, confirmed the importance of certain peak urinary levels of platinum in the pathogenesis of the renal lesion. Furthermore, because certain xenobiotics are handled by the kidney via the same mechanism used for anion transport (13) and since related compounds also inhibit ATPase (16), the platinate inhibitory actions on PAH transport led us to the discovery that certain analogs are potent inhibitors of this enzyme system. These ATPase data certainly suggest that the platinum compounds tested are specific Na,K-ATPase inhibitors with similar modes of inhibition in the fish and mammalian species tested. It is not clear whether this enzyme inhibition in tissue homogenates is related to the observed effects on PAH transport. First, the functional relationship between the enzyme and the transport processes has not yet been determined. Second, we do not know if the enzyme in-

hibition reported here will be found when whole tubules are exposed, ie, does enzyme inhibition occur at sites that are accessible in intact tubules? The present data do indicate, however, that these platinum compounds affect two important membrane-mediated processes in the flounder proximal tubule and that other related possibilities, ie, inhibition of fluid or nutrient transport, should be investigated.

Furosemide is used to enhance the urinary clearance of *cis*-platinum, and since it reportedly inhibits tubule ATPase, the potentiation of nephrotoxicity in our in vitro system (*see* above) was anticipated. Additionally, the fact that this diuretic also causes hearing loss (17) suggests a common mechanism of toxicity for furosemide and platinates at the ATPase level. Thus, in animals, it has been postulated (17) that furosemide inhibits the activity of those ATPases in the ear which are responsible for maintaining the unusual fluid electrolyte concentrations of the endolymph (ie, K^+ = 144 mEq/liter and Na^+ = 16 mEq/liter) (18). Under such conditions the inhibition of active transport of sodium into the adjacent capillaries would lead to sodium accumulation in this fluid compartment resulting in water retention and edema. Such hair cells and renal tubular cells may demonstrate the characteristic subcellular alterations which are reported by Trump et al (19) to result from the movements and redistributions of ions and water in various subcellular compartments. Using a renal slice model, these workers (19) also pointed out that such changes are readily reversible if ion imbalance is restored promptly.

While we feel we have good evidence that platinate toxicity is mediated via inhibition of ATPase in both the kidney and the inner ear, two other target organs of platinate toxicity, peripheral nerves and the gastrointestinal tract, offer indirect clues that the same enzymes may be involved. In his review, Ronquist (20) points out the importance of ATPase for nerve action and for the gastric secretion of HCl. In fact, Mozsik (21) found that human gastric mucosa was relatively rich in ATPases. Thus, we offer as a hypothesis that all four major sites of toxicity, kidneys, inner ear, nerves, and gastrointestinal tract, may involve inhibition of ATPase by *cis*-platinum. Considerable additional work in these laboratories will be directed toward further tests of this hypothesis. In light of recent work, this does seem reasonable since other heavy metals act on such membrane transport and enzyme systems (22). On the other hand, because the PAH transport studies involve the use of relatively intact tubules, these studies hold more promise as a screening tool because they provide an organ-function test and are closer to the whole animal. Platinate inhibition of the semipurified ATPase enzyme system may be useful as a screen but has the potential for the introduction of many more artifacts than the tubule function tests, since so many membrane barriers are destroyed in this preparation.

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Disposition and Distribution of Platinum Following Parenteral Administration of *cis*-Dichlorodiammineplatinum(II) to Animals

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SUMMARY

After iv administration of *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) to animals, plasma levels of platinum decline in a biphasic manner, with a distribution phase (α) half-life of minutes and an elimination phase (β) half-life of days. Urinary excretion of platinum is extensive on the first day after drug administration with a final urinary recovery of 70%–90% of the administered dose. Platinum is initially distributed to nearly all tissues with the highest levels appearing in kidney, liver, ovary, uterus, skin, and bone. There is no preferential uptake of platinum into tumor, although the presence of a tumor may alter the rate of platinum excretion and the extent of whole-body retention. No effect is seen on hepatic microsomal drug metabolism after ip administration of *cis*-platinum to rats. Platinum is excreted more rapidly from hydrated animals than from controls although total urinary recovery of platinum is nearly equal in both groups. Most analogs of *cis*-platinum appear to follow the same elimination and distribution patterns as *cis*-platinum itself.

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Knowledge of the tissue or organ localization of a drug can assist in understanding likely sites for potential toxic or therapeutic effects. Clinical use of a drug can be optimized after blood levels and excretory patterns of the drug have been determined in animals and patients. The overall disposition of *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) and its analogs in animals is only now being fully appreciated, and this review will examine the uptake, distribution, clearance, and metabolic interactions of this drug and its analogs following parenteral administration to animals.

A major difference encountered in many studies of *cis*-platinum disposition is in the analytic methodology utilized. Two methods predominate, although others have been used. One method is atomic absorption (AA) spectroscopy utilizing the flameless graphite atomizer. In order to analyze tissue levels of platinum, however, a cumbersome and potentially hazardous wet digestion technique must be employed. The great sensitivity of this method is exceeded only by the widely recognized sensitivity of radioactive tracer experiments. The major drawback to the routine use of platinum radioisotopes is the short half-life of the β-emitting isotopes and the relative hazard of and equipment needs necessary for use of γ-emitting isotopes. Both the AA spectroscopy and isotopic methods are widely used, however, in the field of platinum disposition. Furthermore, both methods assume that either radioactivity or elemental platinum content is synonymous with the parent drug. This assumption precludes metabolism of the parent molecule and although no work on the metabolism of *cis*-platinum in biologic systems has yet been published, there is convincing presumptive evidence that the dichlorodiammine-substituted molecule does undergo some change after solubilization and subsequent administration to animals. The presence of either elemental platinum or a platinum radioisotope, however, certainly represents the nucleus of the parent drug and that portion of the drug which may be responsible for the majority of the toxic and therapeutic effects.

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PLASMA LEVELS AND URINARY CLEARANCE

Plasma levels and whole-body retention of platinum following administration of *cis*-platinum are similar in all species studied. Plasma levels following iv administration decline in a biphasic manner, with a rapid distribution phase (α half-life of minutes) followed by a prolonged elimination phase (β half-life of days). Urinary levels of drug increase dramatically within the first several hours after drug administration and decline thereafter, with small amounts of platinum detectable in rat urine 30 days after a single dose of drug (*see* fig 1). Total recovery of platinum in mammalian urine is in the range of 70%–90%, most of which appears within the first several days.

The specific plasma half-lives and clearance rates differ quantitatively according to the animal species, dose, route of administration, time points analyzed, and analytic method used. Hoeschele and Van Camp (1), using the rather general method of whole-body scintillation counting, estimated a half-life of 1.6–5 hours for *cis*-platinum in the mouse. Litterst et al (2) initially estimated the α half-life to be 43 minutes and the β half-life to be 2 days for the rat. Our more recent investigation of plasma levels and urinary clearance of platinum in the rat, however, has led to a re-evaluation of platinum kinetics. As shown in table 1, when more numerous time points are studied and fit to a two-compartment model, an α half-life of 9.7 minutes and a β half-life of 35.7 hours are obtained. This α-phase value is in agreement with the value from Wolf and Manaka (3) who found an α half-life of 8 minutes and a β half-life of nearly 4 weeks in the rat. The accuracy of their α half-life was verified using an extravascular blood loop, which gave a value of about 6 minutes. The discrepancy between the β half-lives may be related to methodology. Wolf and Manaka (3) extrapolated to long time points using a radioisotope with a half-life of only 4 days and sacrifice times of only 72 hours after treatment. Litterst et al (2) killed rats for 12 days after treatment and analyzed for platinum using AA spectroscopy. The relative results, however, are qualitatively similar and demonstrate an extremely rapid α phase followed by a less rapid β phase. This same relative relationship between distribution and elimination has also been seen in the dog (2), which has an α half-life of 22 minutes and a β half-life of 5 days, and the dogfish shark (2), which has an α half-life of 71 minutes and a β half-life of 17.5 days (table 1).

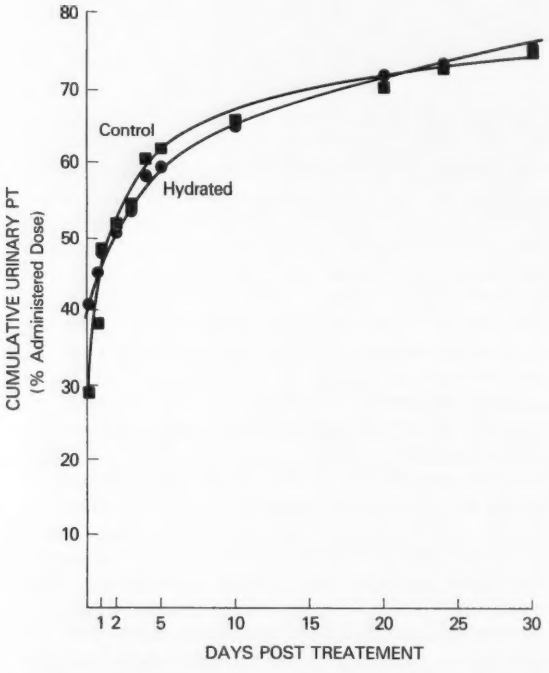

FIGURE 1.—Urinary excretion of platinum in control (■) and hydrated (●) rats for 30 days after iv *cis*-platinum administration (5 mg/kg).

Seventy-nine percent of an ip dose of *cis*-platinum was excreted in mouse urine on the first day after treatment and only an additional 8% on the second day after treatment (4). Similarly, 88% of an ip dose of *cis*-platinum was excreted in mouse urine within the first 5 days after treatment and only an additional 4% of the dose was excreted in the next 10 days (1). The 24-hour urinary recovery of *cis*-platinum following iv dosing of tumored rats was 50%; no comparable data were presented for untumored rats (3). Other investigators (2) have reported a 50% urinary recovery of platinum 4 days after iv administration to rats. More recent data demonstrate about 75% of the total dose recovered in urine of rats in 30 days (fig 1). A 60% recovery of platinum in dog urine within the first 4 hours after iv treatment has been reported (5), and other investigators (6) have recovered 76% of the administered dose 48 hours after iv treatment in the dog.

An interesting finding was a 6-day urinary platinum recovery of only 10% following iv administration of *cis*-platinum to the dogfish shark (2). This finding raises the possibility of either extremely prolonged whole-body retention of platinum in marine species or another route of excretion in these animals. The latter is most likely since high extracellular chloride levels favor the persistence of *cis*-platinum in the extracellular compartment and since it has been pointed out (2) that the chloride level of shark plasma is more than three times that seen in most mammals, including man. Low levels of platinum appear in dogfish shark bile (*see* table 2), and the extremely long β half-life (17.5 days) in this species suggests a long whole-body retention. Excretion through the gill into the aqueous medium has not been investigated

TABLE 1.—α and β half-lives reported for *cis*-platinum by several authors

Species	Half-life α	Half-life β	Comment	Ref No.
Mouse	1.5–5 hrs		Whole-body scintillation counting	1
Rat	5.9 mins	15.6 hrs	Dynamic blood loop	3
	8.6 mins	44.9 days	Animals killed serially	
	16.1 mins	6.6 days	Tumored rats; animals killed serially	
Rat	9.7 mins	35.7 hrs	Control rats; animals killed serially	This study
	12.0 mins	22.9 hrs	Hydrated rats; animals killed serially	
Dog	22 mins	5 days	Cannulated	5
Rat	43 mins	2 days	Animals killed serially	2
Shark	71 mins	17.5 days	Animals killed serially	This study

and is not likely due to the low lipid solubility of *cis*-platinum. The relatively high concentrations of platinum found in the gill (table 2) probably reflect the abundance of –SH groups in this tissue, as is the case with the rectal gland.

Careful evaluation of fecal versus urinary excretion of platinum has not been done following administration of *cis*-platinum. Platinum has been recovered in dog bile (5), and a rate constant for the clearance of platinum via the bile in dogs has been reported (7).[3] Six days after administration of either of two analogs of *cis*-platinum to dogs, substantial amounts of platinum were found in the gall bladder (8), and 13% of an ip dose of the ethylenediammine analog was excreted in the feces (9). In addition, numerous authors have reported low to moderate (2%–7% of administered dose) levels of platinum in various segments of the intestines of animals treated parenterally with *cis*-platinum (5) or some of its analogs (9,10), so the potential for fecal excretion is present. Furthermore, only one group has reported platinum recoveries close to 100% of the administered dose (6), and part of the unrecovered drug may have appeared in the feces. Excretion of ^{191}Pt following iv administration of $^{191}PtCl_4$ to rats occurred in both urine and feces in approximately equal amounts (11). The differences between results with *cis*-platinum and its analogs might well be related to differences in water solubility, lipid/water partition coefficients, or metabolic alterations between the parent *cis*-platinum and the various analogs or salts (7).

Perhaps the most significant finding in the past several years in the field of platinum drug use was the discovery that prior hydration and/or diuresis could reduce the serious renal toxicity of *cis*-platinum. Plasma levels of platinum were found to be equal in both mannitol-treated and prehydrated dogs (6), although comparative curves for dogs receiving platinum alone were not presented. Urinary platinum concentrations, however, were greater for prehydrated dogs than for control dogs (platinum alone) and less than controls for mannitol-treated dogs. Because the total 48-hour urinary recovery of platinum was 90%–100% for both mannitol-treated and prehydrated dogs, the changes in urinary platinum concentration must be related to different total urine volumes in the two treated groups. The total 48-hour urinary recovery of platinum in dogs treated with *cis*-platinum only was 76%. Our recent work (fig 2) has shown that plasma levels of platinum are not different from those in controls in rats hydrated with normal saline before and after *cis*-platinum administration. Urinary recovery of platinum was significantly greater at early times in the hydrated rats (fig 3). This difference disappeared after the second day, however, and recoveries were equal in the two groups from 2 to 30 days after treatment (fig 1). In contrast to data on prehydrated dogs (6), these experiments showed a decreased urinary platinum concentration in hydrated rats which correlated with significantly greater urinary volume during the 2 days of hydration (data not presented).

ORGAN DISTRIBUTION

All animal species studied to date demonstrate the same general organ distribution of drug. The drug undergoes initial distribution to nearly all tissues, followed within the first hour by an accumulation of the drug in a few selected organs (kidney, liver, muscle, and skin). By the end of the first day, rapidly falling plasma levels produce tissue:plasma drug ratios of > 1 in numerous

[3] In addition, since the submission of this manuscript a report has appeared (26) which shows that < 2% of an iv dose of ^{195m}platinum administered as *cis*-platinum is excreted in the bile of bile-duct cannulated rats during the 1st 24 hrs after treatment.

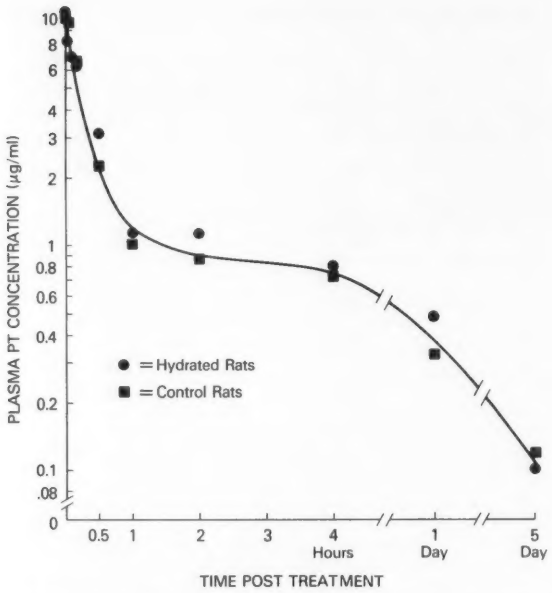

FIGURE 2.—Plasma concentration of platinum (PT) in control (■) and hydrated (●) rats after iv *cis*-platinum administration (5 mg/kg).

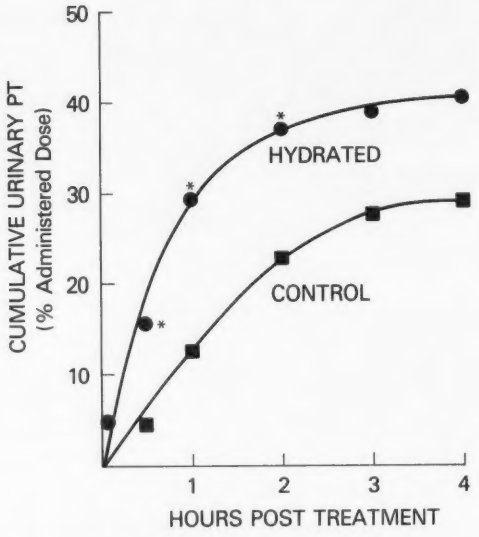

FIGURE 3.—Urinary excretion of platinum (PT) in control (■) and hydrated (●) rats during the 1st 4 hrs after iv *cis*-platinum administration (5 mg/kg).

other tissues. These elevated tissue:plasma ratios are maintained for at least 1 week in the dog and probably other species. By 2–4 weeks after treatment, significant levels of platinum can still be found in kidney, liver, skin, and lung.

Ten minutes after iv administration of *cis*-platinum (1 mg/kg) to the dog (5) nine of 15 tissues studied had platinum concentrations of > 1 μg/g, with kidney and plasma having the highest concentrations (6.6 and 2.6 μg/g, respectively) and spleen, pancreas, intestine, heart, and muscle having the lowest concentrations (0.3 μg/g). By Day 12 after treatment, the kidney and liver still had substantial concentrations of platinum (0.6 and 0.3 μg/g, respectively) and muscle and skin had detectable concentrations (0.04 and 0.2 μg/g, respectively). In the rat (2), the kidney and liver had high concentrations of platinum (9 and 3 μg/g, respectively) 1 hour after treatment. When tissue mass is considered (3,12) however, skin, bone, muscle, and liver have the greatest absolute amounts of platinum (16%, 6.5%, 5.7%, and 4.8% of administered dose, respectively) 1 hour after treatment. These same relative ratios are maintained for at least 3 days after treatment (3,12).

A whole-body scanning device showed that a generalized distribution of radioactivity after iv administration of *cis*-platinum to rabbits is followed by an accumulation of label in liver, kidney, and bladder 40 minutes after treatment (4). When rabbits were killed 18–24 hours after treatment, the kidney and liver contained significant quantities of drug (tissue:plasma ratios of 12.5 and 5.6, respectively). Most tissues, including bone, contained 1.5–2.5 times the blood level of platinum. Of interest, however, was a tissue:blood ratio of 4 for skull, even though brain tissue contained only small amounts of drug. Similar work 2 hours after ip treatment in the mouse (4), however, showed that both the skull and rib contained amounts of radioactivity no greater than those in the blood.

Distribution of platinum in the female dogfish shark (table 2) is similar to that seen in mammals, with the highest levels occurring in kidney and uterus. High concentrations are also present in the heart, rectal gland, and duodenum. The liver contains only moderate levels of platinum, as distinct from the high concentrations seen in mammalian liver, although the levels are constant for at least 6 days after treatment.

As early as 2 hours after treatment small amounts of platinum are present in brains of tumored mice treated ip with *cis*-platinum (1). Lange et al (13) found small amounts of platinum in brains of both rabbits and tumored mice 18 or 24 hours after treatment and suggested that residual blood in the mouse brain would account for the amounts of platinum detected there. Platinum brain concentration in rats was nearly constant (0.06% of administered dose) for the first 24 hours after treatment (3). This relatively constant concentration of drug is higher than would be expected from residual blood because blood levels of platinum drop dramatically in the first day after treatment (2,3,5). By 72 hours after treatment brain levels had decreased to 0.02% of the dose (3),

TABLE 2.—Distribution of platinum at various times after iv administration of *cis*-platinum (1 mg/kg) to female dogfish sharks *(Squalus acanthus)**

Tissue	Platinum concentration at post-treatment times of—								
	10 mins	30 mins	1 hr	2 hrs	4 hrs	24 hrs	2 days	4 days	6 days
Plasma	8.52	8.06	4.49	3.47	1.74	0.77	0.69	0.62	0.66
Urine	0.38	6.83	20.61	46.03	160.0	3.35	4.67	9.53	18.32
Cerebrospinal fluid	0.11	0.16	0.30	0.41	0.48	0.18	0.27	0.32	0.30
Brain	0	0.38	0.24	0.42	0.25	0.83	0.75	0.85	0.70
Bile	0	0	0	0.25	0	0.28	0.46	6.17	4.62
Liver	1.13	—	—	0.77	1.10	1.07	1.24	1.86	1.20
Pancreas	2.65	1.11	1.24	2.21	1.36	1.26	1.40	1.37	1.44
Spleen	3.74	1.41	1.77	1.26	1.26	1.23	0.96	1.58	0.94
Uterus	5.46	4.06	4.29	3.94	2.70	2.89	2.97	2.52	1.95
Rectal gland	5.39	3.28	2.98	3.31	4.62	2.90	1.83	2.39	1.72
Kidney	9.03	5.71	6.31	5.23	6.00	4.93	3.82	6.26	5.15
Duodenum	2.89	3.53	3.00	4.78	3.30	2.19	1.74	4.05	2.06
Heart	5.16	2.48	3.40	4.00	2.06	1.81	1.58	1.27	1.28
Muscle	0.51	0.26	0.91	0.71	1.01	1.01	0.52	0.81	0.51
Gill	2.09	3.20	1.80	0.46	0.25	0.61	0.54	0.37	0.74

*Data are the average concentrations of *cis*-platinum/ml or g from 2 fish, except on Day 6, where 6 fish were used.

and 4½ days after injection of *cis*-platinum to mice negligible amounts of platinum remained in the brain (1). In the dogfish shark (table 2) brain concentrations of platinum are constant for the first day after treatment and then increase two to three times to levels which remain constant for the next 6 days. At 1, 2, 4, and 6 days after treatment with *cis*-platinum brain concentrations of platinum in the shark are equal to plasma concentrations, suggesting that there is a small pool of platinum either tightly bound to brain tissue or unable to exit through the blood-brain barrier. Although there are substantial differences between the central and peripheral nervous systems, the presence of platinum in the central nervous system might be relevant to recent reports of peripheral neuropathies in patients receiving *cis*-platinum (14). In addition, ATPases play an important role in nerve tissue and the inhibition of these enzymes by platinates has recently been shown (15).

Localization of platinum in bone has also been reported. Six percent to 8% of an injected dose was present in rat bone during the first 3 hours after treatment and levels declined slowly to 3.5% by 72 hours (3). Only skin contained more drug residue than did bone, and muscle contained an amount of radioactivity equal to that found in bone. It was postulated (3) that these tissues of large mass might represent a reservoir for the release of platinum during its long clearance times. In support of this theory, it was found (5) that low but stable concentrations of platinum occurred in muscle of dogs for up to 6 days after an iv dose of *cis*-platinum, and skin concentrations of platinum were similar to concentrations in kidney, liver, and lung at 4 days after treatment. Platinum was maintained at significant concentrations in the skin for at least 12 days.

No consistent localization of drug in tumor tissue has been found. Wolf and Manaka (3) found no significant concentration of platinum in Walker 256 tumors in rats over a 72-hour time course, and there was no specific uptake of platinum into the Sarcoma 180 tumor in mice (1). Lange et al (13) however showed a constant tumor:plasma ratio of platinum of nearly 2 for all times from 4 hours to 5 days after treatment for Sarcoma 180-bearing mice. When drug dose was increased by a factor of ten, no significant change in tumor:plasma ratio of platinum was evident at 4 or 24 hours but a slight increase was seen 5 days after treatment. Of significance in this latter study (4) was an increased whole-body retention of platinum in tumored versus control mice that could be related to platinum retained in the tumor. Other authors have also reported different organ distributions or whole-body clearances between tumored and normal animals. Thus, organ levels of platinum in Sarcoma 180-tumored mice were consistently higher than levels in nontumored mice 4 days after treatment (1). Wolf and Manaka (3) reported an α half-life for platinum that was nearly twice as long in Walker 256-tumored rats as in controls, but a β half-life that was only about one sixth as long (table 1). These results are doubly intriguing when viewed in light of their finding of no significant localization of platinum in tumor tissue (3). Sarcoma 180-tumored mice excreted platinum significantly more slowly and retained higher amounts of platinum than did their nontumored controls (1). Forty-eight hours after iv administration of *cis*-platinum to Walker 256-tumored rats, there was a general decrease in organ concentration of platinum in tumored rats relative to controls (12), reflecting perhaps the increased clearance of platinum in these animals. Thus the tumored:control ratio of platinum in plasma was 0.5 and tissue levels ranged from 0.2 for the adrenal gland to 0.6 for the kidney. The tumored:control ratio of platinum in all other tissues was 0.2–0.3. This decrease in platinum concentration in tissues from tumored rats was

not observed, however, at 24 or 72 hours after treatment (12).

The functional integrity of the kidney is of concern because of the major role the kidney plays in drug elimination. When early, very high urinary levels of a drug are widely reported, it becomes of major importance to assure normal kidney function when studying pharmacokinetics, particularly when the drug (*cis*-platinum) is known to be nephrotoxic. Few preclinical studies have apparently monitored kidney function in experiments where *cis*-platinum disposition was investigated in animals. Thus, serum BUN and creatinine levels were monitored in dogs (5) and rats (2) and no significant changes from normal were found during the 12 days of these studies, and BUN and creatinine levels were normal in dogs during the 48-hour course of other experiments (6).

The prolonged retention of platinum in some organs suggests an irreversible or very tight binding to tissue components. The in vitro binding of platinum to dog plasma was altered as a function of time, with an increasing amount of drug retained in the plasma as the time of exposure was prolonged (5,16,17). The nature of the plasma binding component has been addressed by several authors. Thus, one report (5) showed no association of platinum with the cellular elements of blood, in essential agreement with results (18) demonstrating that the dipyridine analog of platinum was not associated with erythrocyte membranes. Platinum binding to bovine albumin in vitro parallels platinum binding to dog plasma when *cis*-platinum is used (5), although no binding of *cis*-platinum analogs to albumin has been reported (10,18). In a recent investigation into distribution of platinum in blood following *cis*-platinum, 89% of the platinum localized in the plasma fraction and 11% localized in the rbcs 2 minutes after administration, but only 12% was in the plasma 72 hours after administration (17). During the first hour 5% of the dose in the rbcs was associated with the ghosts. Five minutes after administration, 40% of the drug could be recovered with globulin and presumably 60% with albumin and other plasma proteins. Forty minutes after treatment, however, 40% was associated with the albumin fraction, suggesting a shift in association of platinum with plasma macromolecules.

Details of the subcellular distribution of *cis*-platinum have not been published but localization of *cis*-platinum in the cytosol of homogenized tissues has been alleged (19). This is in contrast to other results where only small quantities of platinum were found outside the nucleus of tissue culture cells 4 hours after treatment with *cis*-platinum (20). This suggests a possible relocation of platinum from the nucleus at early times after treatment to other cell fractions at later times. Twenty-four hours after ip administration of the ethylenediammine analog of *cis*-platinum to rats, there was no specific association of platinum with any of five subcellular fractions from liver or tumor (21). In the kidney, however, there was a strong association of drug with the microsomal fraction. The lack of association of platinum with microsomal membranes of liver is supported by a lack of inhibition by *cis*-platinum of hepatic microsomal drug-metabolizing enzymes (table 3). In these experiments *cis*-platinum was injected ip once, or in four daily injections. Hepatic microsomes were then prepared and the drug-metabolizing enzyme system in control and treated rats was characterized by standard methods (22). This lack of platinum effect on microsomal drug metabolism is consistent with other reports (23). In the multiple-dose study, the specific activities of *N*-acetyltransferase, glutathione transferase, and NADPH cytochrome c reductase were all increased by *cis*-platinum treatment. The increase in enzyme activities is consistent

TABLE 3.—Effect of ip *cis*-platinum on in vitro hepatic drug metabolism following a single dose or 4 daily doses to Sprague-Dawley rats*

	Drug-metabolizing enzymes							
cis-Platinum	Cytochrome P–450†	Cytochrome c reductase‡	Aniline hydroxylase‡	Aminopyrine demethylase‡	*para*-Amino benzoic acid *N*-acetyl-transferase‡	UDP glucuronyl-transferase‡	Glutathione S-aryl transferase‡	Total heme†
				Males				
Single dose								
Control	0.105 ± 0.012	137 ± 9	1.56 ± 0.16	6.01 ± 1.15	0.73 ± 0.06	3.54 ± 1.09	79.3 ± 11.1	0.051 ± 0.006
5 mg/kg	0.085 ± 0.008	143 ± 10	1.85 ± 0.33	6.05 ± 0.39	0.54 ± 0.12§	3.14 ± 1.03	96.8 ± 16.5	0.049 ± 0.003
10 mg/kg	0.088 ± 0.008	146 ± 12	1.87 ± 0.20	7.03 ± 1.23	0.90 ± 0.11§	3.06 ± 0.55	94.1 ± 19.0	0.053 ± 0.004
				Females				
Daily × 4 dose								
Control	0.063 ± 0.002	194 ± 12	0.61 ± 0.06	2.60 ± 0.49	0.45 ± 0.13	6.75 ± 1.09	42.5 ± 2.4	0.037 ± 0.002
4 mg/kg/day	0.057 ± 0.005	237 ± 16§	0.55 ± 0.09	1.94 ± 0.29	0.85 ± 0.11§	6.54 ± 1.50	51.0 ± 2.8§	0.034 ± 0.004

*Data are means ± SD; No. = 4.
†Change in optical density/mg of protein.
‡nmols/mg of protein/min.
§$P \leq 0.05$, treated vs control.

TABLE 4.—Effect of in vitro addition of *cis*-platinum or chloroplatinic acid on the in vitro demethylation of aminopyrine in hepatic microsomes*

Platinum concentration (μM)	*cis*-Platinum	Chloroplatinic acid
0	8.79	8.11
1	8.82	8.30
5	9.40	6.47
10	9.02	4.71
50	8.86	0.05†

*Data are expressed as the average specific activity (nmols/mg of protein/min) of 2 determinations. Microsomes were prepared as described in ref 22.

†pH of the incubation mixture containing 50 μM of platinum as chloroplatinic acid was 5.1. pH of the incubation mixtures containing all other concentrations of chloroplatinic acid and all concentrations of *cis*-platinum was 7.3 ± 0.1.

with the fact that sometimes heavy metals stimulate enzyme activities. The reproducible alteration in the activities of transferases localized in the cytosol may reflect the presence of platinum in the cytosol (21). In contrast, ip administration of $PtCl_4$ produced an increase in hexobarbital sleeping time and a decrease in oxidative drug metabolism in liver microsomes (24). Chloroplatinic acid, another inorganic platinum salt, similarly has been shown to be more toxic than *cis*-platinum both in vivo (25) and in vitro (*see* table 4 and ref 15).

Tissue localization of platinum might be expected to provide a clue to potential sites of toxic reactions. The high and sustained kidney levels of platinum following *cis*-platinum administration suggest an obvious correlation between platinum renal toxicity and the organ localization of the drug. An exception was the lack of platinum retention in animals dying either early or 5 or 6 days after an ip injection of *cis*-platinum (1). Bone marrow also represents a site of *cis*-platinum toxicity but negligible amounts of platinum were found in the marrow, even though the bone matrix was found to contain significant concentrations of platinum during the first 0.5–72 hours after treatment (3). A similar result was found for bone marrow and bone matrix using the ethylenediammine analog (21). The small amounts of platinum reported in brain tissue by several authors (1,3,13), however, suggest a potential correlation with a centrally mediated emetic response and, as suggested earlier, with peripheral neuropathy.

It might also be reasonable to expect a correlation between organ distribution of *cis*-platinum and organ site of antitumor effect. *cis*-Platinum, as a single agent in the treatment of human neoplasms, is active primarily against tumors of the ovary and testes. High levels of platinum were found in the ovary and uterus of the dog following iv *cis*-platinum administration (5). However, only low levels of platinum have been reported in the testes of rats following iv administration of *cis*-platinum (2,3) or its ethylenediammine analog (21).

ANALOGS OF *CIS*-PLATINUM

Several analogs of *cis*-platinum have been investigated for their distribution and elimination characteristics (1,8–10,18,21,25). Much of the data on these analogs are compromised by abbreviated studies, and the only analogs investigated are those discovered relatively early in the development of this drug class. No information is available on numerous, more recently investigated analogs. It appears that all analogs studied to date demonstrate the same distribution and elimination patterns as does the parent drug. Kidney and liver have the highest concentrations of drug (8–10,25) and large amounts of drug are localized in skin (10) and muscle (8). The analogs appear to be excreted primarily in the urine (9,10,25), although the rate and possibly the total amount excreted may be less than for *cis*-platinum. At least two of the analogs have been found in bile (8). Some analogs localize in tumor tissue (21) while others do not (25).

CONCLUSION

In conclusion, platinum-containing antineoplastic agents exhibit a biphasic plasma clearance. The extremely rapid α phase accounts for early, high levels of platinum in kidney, liver, skin, bone, ovary, and uterus. The prolonged β phase is reflected by urine concentrations of platinum that are still detectable 30 days after a single dose. The drug is excreted primarily in the urine, although a small amount of fecal excretion may occur. Total urinary recoveries are 70%–90% of the administered dose in most mammals. Drug concentrations are highest in liver and kidney. Moderate concentrations of drug are found in skin, muscle, and bone. The large relative mass of these latter tissues and their prolonged retention of platinum recommend these tissues as reservoirs which account for the long β half-life of this drug. Little drug is found in tumor but the presence of tumor may alter the distribution and excretion pattern of the drug. Physiologic manipulations of animals such as hydration can increase the early rate of platinum excretion.

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Rationale for Development of Platinum Analogs [1]

Joseph H. Burchenal,* Kathleen Kalaher, Kimberly Dew, and Linda Lokys [2]

SUMMARY

There is a great need for clinical trial of new second-generation platinum coordination compounds that might demonstrate greater clinical activity in a broader spectrum of tumors, decreased renal toxicity and emesis, improved solubility, synergism in combination therapy, and lack of cross-resistance to *cis*-dichlorodiammineplatinum(II) (*cis*-platinum). Lack of cross-resistance to *cis*-platinum is shown by certain 1,2-diamino-saturated cyclic platinum derivatives which also have a high degree of activity against transplanted mouse leukemias. *cis*-Platinum and these cyclic compounds combine synergistically with derivatives of cytosine arabinoside, VP-16-213, and Adriamycin. These 1,2-diamino cyclic compounds appear to have less renal toxicity than *cis*-platinum. The toxic and therapeutic effects of both *cis*-platinum and the diamino cyclic compounds can be blocked by massive doses of thiourea. Varying doses and time intervals of thiourea rescue are being studied in the hope of improving the therapeutic index of the platinum derivatives.

[Cancer Treat Rep 63:1493–1498, 1979]

cis-Dichlorodiammineplatinum(II) (*cis*-platinum) (1,2) is a first-generation platinum coordination compound that has therapeutic activity against a wide spectrum of human tumors (3,4), both alone and in combination with other agents. Since some tumors are totally refractory to *cis*-platinum, and since even in most cases responding to treatment tumor resistance or nephrotoxicity does eventually develop, large numbers of compounds have been tested both alone and in combination against transplanted animal tumors and leukemias (5–8) and some appear to be considerably more active than *cis*-platinum. For instance, the early work of Connors et al (5) using a plasma cell tumor showed an advantage for compounds containing bis-cyclic amino over unsubstituted diammine groups which culminated in the bis-cyclopentyl amine and bis-cyclohexyl amine platinum dichlorides which had therapeutic indices roughly 20–25-fold greater than *cis*-platinum. Despite this outstanding therapeutic activity against plasma cell tumors in mice, however, no such advantage was seen with these compounds against L1210 leukemia. We have screened many other platinum coordination compounds, particularly the 1,2-diamino cyclic derivatives, both alone and in combination against transplanted L1210 and P388 mouse leukemias sensitive to and resistant to *cis*-platinum (8).

The rationale for the search for newer compounds of this series would be to find compounds with (*a*) a lack of cross-resistance to *cis*-platinum which theoretically might result in therapeutic activity in patients whose disease has become resistant to the antitumor effects of *cis*-platinum; (*b*) a broader spectrum of activity, particularly in clinical areas where *cis*-platinum has not demonstrated significant therapeutic effect; (*c*) greater clinical antitumor effectiveness; (*d*) decreased renal toxicity and emetic effects which, in many cases, limit the amount of *cis*-platinum which can be tolerated; (*e*) improved solubility, particularly for use by the intravesical, ip, or intra-arterial route; and (*f*) synergism in combination therapy with other antitumor agents and, if possible, an ability to block the toxic but not the therapeutic effects of high doses of these platinum derivatives by thiourea (TU) or other sulfur-containing compounds.

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METHODS

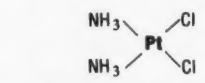
The technique for evaluating the chemotherapeutic activity of a drug by its ability to prolong the survival time of mice with transplanted leukemias has been reported previously (8). The experiments described here were done with the L1210, P388, and L5178Y mouse leukemias and their respective sublines resistant to cytosine arabinoside, methotrexate, or *cis*-platinum in (C57BL/6 × DBA/2)F_1 mice. L1210/*cis*-platinum and P388/*cis*-platinum are the L1210/0 and P388/0 leukemia lines that were treated with single 8-mg/kg doses of *cis*-platinum 24 hours after inoculation of the leukemias over successive generations until no significant increase in survival time was seen with any tolerated dose of *cis*-platinum (8,9). In all experiments, 1,000,000 leukemic cells suspended in 0.1 ml of 0.85% NaCl solution were inoculated ip into each animal, producing an ascitic leukemia that later progressed to the generalized disease. The mice were divided into groups of ten animals each, and treatment was initiated 24 hours to 6 days after the inoculation of leukemic cells and continued once every fourth day (Days 1, 5, 9, and 13). Compounds were dissolved in 0.85% NaCl solution or sterile H_2O and injected ip unless otherwise noted.

For cell culture studies, a modification (8) of the technique of Fischer et al (10,11) was used. The cells were incubated in McCoy's medium with 15% fetal calf serum. The initial inoculum was 40,000–60,000 leukemic cells/ml. For growth-inhibition studies, 0.1 ml of a 50-fold concentration of the drug in question was added to 5 ml of the cell-containing medium. The tubes were set up in groups of four, loosely capped, and allowed to incubate in 5% CO_2 at 37°C for 96 hours. Growth to approximately 10^6 cells/ml occurred in the control tubes. The contents of each tube were agitated to resuspend the cells and counted in a Coulter counter. The percentage of growth inhibition and the doses inhibiting 50% of cell growth were calculated. Cell culture experiments were done with L1210, L1210/*cis*-platinum, and L5178Y mouse leukemia cell lines.

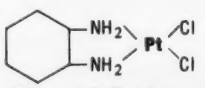
RESULTS

Of the compounds tested so far, only the 1,2-diamino cyclic derivatives of *cis*-platinum have shown a lack of cross-resistance to *cis*-platinum. Substitutions on the anionic side may affect toxicity, solubility, and, to some extent, therapeutic index, but so far have had no effect on this cross-resistance or lack thereof. The dichloro, malonato, sulfato, and carboxyphthalato derivatives of 1,2-diaminocyclohexane (DACH) (fig 1) and 1,2-diaminocycloheptane (DACHE) platinum are equally effective in vitro against L1210 leukemia and the line made resistant to *cis*-platinum (L1210/*cis*-platinum) as shown in table 1. Surprisingly, in this test system, the 1,2-diaminocyclopentane (DACP) derivatives show a high degree of cross-resistance. Similarly, unsubstituted amine, bis-substituted amine, ethylenediamine, and orthophenylenediamine derivatives show cross-resistance. In vivo, the DACH and DACHE derivatives are again highly active against both the *cis*-platinum-sensitive and *cis*-platinum-resistant L1210 and P388 lines (figs 2 and 3), and, in addition, some activity, although to a lesser degree, is seen in DACP derivatives (table 2). Again, unsubstituted amine, bis-substituted amine, ethylenediamine, and orthophenylenediamine derivatives are inactive against the *cis*-platinum-resistant lines. *cis*-Platinum in maximum-tolerated doses is active against lines made resistant to the DACH compounds (table 3) as are, to an even greater extent, cyclophosphamide, melphalan, vindesine, Adriamycin, and VP-16-213.

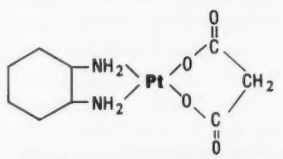
Unfortunately, experimental data are not available to indicate whether these 1,2-diamino cyclic compounds are likely to have a broader spectrum of activity against human tumors. On the other hand, there are data which show that both the malonato- and carboxyphthalato-DACH platinum derivatives have less kidney toxicity in



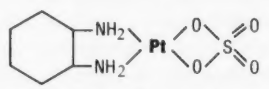
dichloro-diammino Pt (DDP)



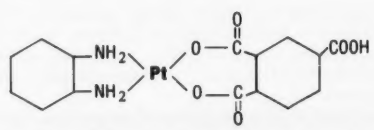
dicloro-1,2 diaminocyclohexane Pt (Pt 155)



malonato-1,2 diaminocyclohexane Pt



sulfato-1,2 diaminocyclohexane Pt



carboxyphthalato-1,2 diaminocyclohexane Pt

FIGURE 1.—Derivatives of DACH.

TABLE 1.—In vitro studies of cross-resistance of platinum coordination compounds (platinum A_2X_2)

A	X	ID50 (μg/ml) L1210	ID50 (μg/ml) L1210/*cis*-platinum
Diammino	Dichloro	0.05	2.50
Diammino	Malonato	0.40	6.50
DACH	Malonato	0.25	0.25
DACH	Dichloro	0.14	0.14
DACH	Sulfato	0.20	0.36
DACH	Carboxyphthalato	0.30	0.23
DACHE	Malonato	0.50	0.09
DACHE	Dichloro	0.06	0.20
DACHE	Sulfato	0.30	0.50
DACP	Malonato	0.60	5.0
DACP	Dichloro	0.30	4.6
DACP	Sulfato	0.60	4.9
Ethylenediamine	Dichloro	0.35	29.0
Orthophenylenediamine	Dichloro	0.69	2.4
bis-Isopropylamino	Dichloro	0.6	4.4
bis-Isopropylamino	Sulfato	0.6	5.3

animals at the LD10 and LD50 doses than does *cis*-platinum (12). This suggests that in patients larger doses could be given, to the point of bone marrow toxicity, and that, therefore, greater effects against the hematologic malignancies might be achieved. The original clinical reports by Hill et al (13) and Ribaud et al (14) on the malonato-DACH platinum derivative suggest less nephrotoxicity, and activity against acute leukemia as well as testicular tumors.

As previously mentioned, improvement in solubility would be highly desirable if the compound were to be used locally in the treatment of intravesicular bladder tumors or ascitic tumors (as in carcinoma of the ovary), or by the intra-arterial perfusion route in the treatment of tumors localized to the liver or to an extremity. The data available suggest that the malonato- and dichloro-DACH compounds are even less soluble than *cis*-platinum, but that the carboxyphthalato compound is highly soluble up to 20 mg/ml (20-fold greater than *cis*-platinum) in a 1% sodium bicarbonate solution.[3]

TABLE 3.—Lack of cross-resistance in malonato-DACH-resistant line (P388/malonato-DACH)

Drug	Dose (mg/kg)	ILS* (%)
Control	—	—
Malonato-DACH	75	+5
Malonato-DACH	50	–22
cis-Platinum	8	+58
cis-Platinum	6	+36
Cyclophosphamide	200	+193
Melphalan	10	+94
Vindesine	0.5	+114
Adriamycin	4	+151
VP-16-213	20	+220

*ILS = increase in lifespan.

We have previously reported several combinations which are highly synergistic with *cis*-platinum (9,15). Similar synergism is seen with the malonato derivatives of both DACH and DACHE and 2,2′-anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine (AAFC) (figs 2 and 4), cytosine arabinoside, VP-16-213, Adriamycin, or methotrexate. These combinations have a high degree of effectiveness and produce a significant percentage of cures in animals injected ip with 10^6 cells of either L1210 or P388 leukemia even when treatment is delayed until Day 4, 5, or 6. At this advanced stage of the disease the single compounds alone have very little effect.

[3]Gale G. Personal communication.

TABLE 2.—In vivo studies of cross-resistance in L1210 leukemia

Compound	Dose (mg/kg) on Days 1, 5, 9, and 13	L1210 Survival (days)	L1210 ILS* (%)	L1210/*cis*-platinum Survival (days)	L1210/*cis*-platinum ILS (%)	L1210/platinum 155 Survival (days)	L1210/platinum 155 ILS (%)
Control	—	8.8	—	12.0	—	12.2	
cis-Platinum	6.7	—	—	12.3	3	18.9	55
cis-Platinum	4.5	14.9	69	13.5	13	16.8	38
DACH dichloro platinum	5.0	20.4	32	22.9	90.8	12.3	1
DACHE dichloro platinum	5.0	13.2	50	18.9	57.5	14.9	22
DACP dichloro platinum	15.0	12.7	44	18.6	55	12.8	5
Dichloroethylenediamine platinum	20	14.3	63	13.2	10		
Dichloroethylenediamine platinum	13	12.8	45	13.7	14		

*ILS = increase in lifespan.

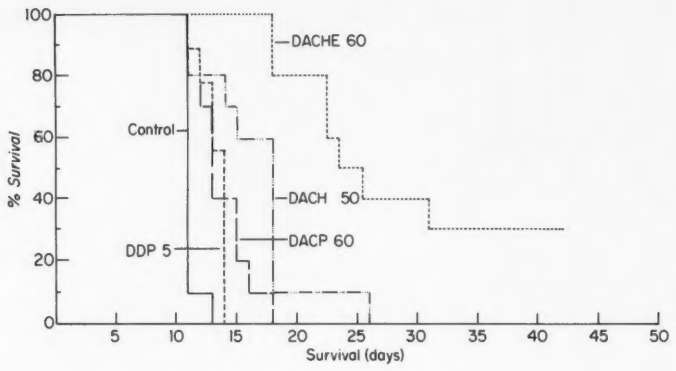


FIGURE 2.—Effects of malonato DACH, DACHE, and DACP derivatives against L1210/*cis*-platinum. Nos. = dose in mg/kg given q4days × 4. DDP = *cis*-platinum.

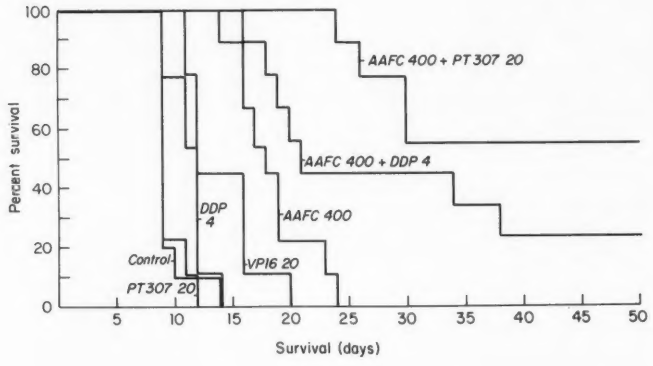


FIGURE 3.—Synergistic effects of AAFC and *cis*-platinum (DDP) or carboxyphthalato-DACH platinum (PT307) in advanced L1210 leukemia. Nos. = dose in mg/kg given on Days 6, 10, 14, and 18.

In in vitro studies on the mechanisms of action of *cis*-platinum, it was found that when TU was added to the medium at the same time as *cis*-platinum, 100 μg/ml of TU would prevent the inhibitory effect of 30 μg/ml of *cis*-platinum (600 times the ID50). Methionine, thiouracil, and sodium thiosulfate also blocked this inhibitory effect in our system, although to a lesser degree than TU, but cysteine did not block this effect at any nontoxic concentration used. When titrated down, approximately a 100-fold molar excess of TU was required to block the inhibitory effects of *cis*-platinum whereas approximately a 300-fold molar excess of methionine was needed (9). If TU was added at this dose level 2, 4, or 24 hours later, however, no reversal of the inhibitory effect was noted. In this regard, however, it is of great interest that at the molecular level Filipski et al (16) have shown that TU not only prevents the cross-linking of DNA by *cis*-platinum, but also *reverses* the *cis*-platinum cross-linking to give a normal DNA when TU is added in massive concentrations (1 M) for 24 hours.

The toxic and antileukemic effects in vivo of 20 mg/kg ip of *cis*-platinum on Days 1, 5, 9, and 13 could be prevented by 800 mg/kg of TU injected 15 minutes before, either ip or sc. Similar prevention of the toxicity of 22.5 mg/kg of DACH dichloro platinum (Pt 155) could be achieved by similarly administered sc doses of TU. In an attempt to increase the overall efficacy of these compounds, smaller but still toxic doses of *cis*-platinum (10 mg/kg q4days × 4) or Pt 155 (15 mg/kg) were given ip with sc doses of TU 15 minutes before (from 800 to 50

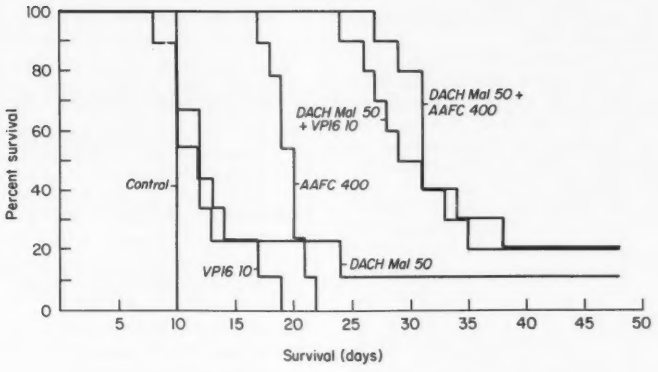


FIGURE 4.—Synergistic effects of malonato-DACH platinum (DACH Mal) and AAFC or VP-16-213 in advanced L1210 leukemia. Nos. = dose in mg/kg given on Days 6, 10, 14, and 18.

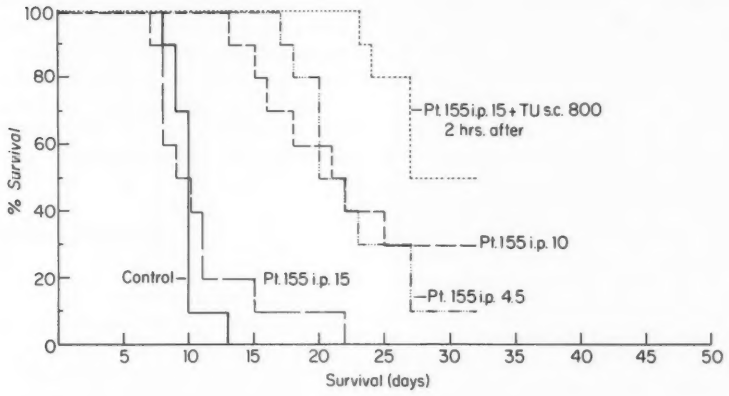


FIGURE 5.—Effect of sc TU on therapeutic activity of large ip doses of Pt 155 in L1210 leukemia. Nos. = dose in mg/kg given on Days 1, 5, 9, and 13.

mg/kg) or 30, 60, 120, and 240 minutes after (800 mg/kg) the platinum derivatives. Possible enhancement of the therapeutic effect was noted by lowering the dose of TU or waiting 60–240 minutes before attempting TU rescue (fig 5), but these were minor increases over the effects of the doses of Pt 155 alone and may have been due to partial blocking of the effects so that 15 mg/kg had the activity of 8–10 mg/kg of Pt 155 alone. These results do suggest, however, that perhaps moderately high doses (LD100) of either *cis*-platinum or Pt 155, with TU or other thio-containing compounds blocking toxicity either immediately before or after (minimal doses) or 1–4 hours later (massive doses), might be superior to any tolerated dose of these compounds used alone. These combinations are now being evaluated against advanced L1210 and P388 leukemias where even maximum-tolerated doses of *cis*-platinum alone are usually only weakly active.

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Structure-Activity Relationships of the Antitumor Platinum Coordination Complexes

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The development of the platinum complexes as antitumor agents has followed the typical pattern of development for most new agents that eventually prove to be of value in the treatment of cancer. Following Rosenberg's initial publication of the effects of *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) in mice bearing sarcoma 180 (1), there was little interest in the potential of this class of chemicals until the experiments had been confirmed and extended by other laboratories (2). Early clinical trials emphasized the toxicity of *cis*-platinum, and it was not until some of the major dose-limiting toxic effects were overcome that it began to be used more widely. The more recent results clearly demonstrating the clinical superiority of *cis*-platinum over other available agents in the treatment of some types of cancer have now led to the world-wide use of this drug in clinical chemotherapy and to the search for more selective congeners.

Structure-Activity Relationships

Following early studies of a number of platinum complexes, some empirical rules were formulated to explain the structure-activity relationships of the series (fig 1). Many chemicals containing platinum have either bacteriostatic or bacteriocidal activity, but it was soon apparent that there was no correlation between the antibacterial properties of a complex and its antitumor activity, therefore precluding antibacterial assays as rapid tests for new complexes. It was also not surprising to find that charged complexes were inactive. It was appreciated quite early that, because of its resemblance to the bifunctional nitrogen mustards, *cis*-platinum might cause cytotoxicity by reacting with DNA. The likely restricted entry of charged complexes into cells would prevent this reaction and, like a number of positively charged alkylating agents that have been studied, these complexes would be expected to be without antitumor properties.

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The importance of the leaving groups and the other ligands was also apparent from the early studies, but it is not essential, as was first thought, that the leaving groups have an optimum lability. It is now known that complexes with an extremely labile leaving group can have antitumor activity; there are also one or two cases where active complexes have extremely stable ligands, implying that some form of activation, enough so that reaction with DNA can occur, must take place in vivo to a complex where the leaving groups are labile.

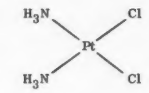
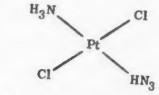
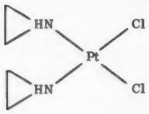
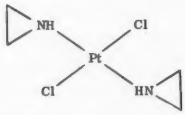
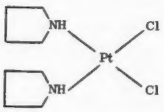
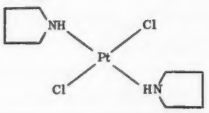
To date, no complex has been found to have antitumor properties when the leaving groups are situated *trans* to one another. Table 1 shows a series of *cis* and *trans* pairs tested quantitatively against the ADJ/PC6 plasma cell tumor (PC6 tumor). Although the *trans* isomers may sometimes be of the same order of toxicity as the corresponding *cis* derivatives and may show the same type of toxicity, they are invariably without antitumor action. In the few cases where *trans* complexes have been reported to be active, such activity has subsequently been shown to be due to traces of *cis* impurity. The stereospecificity of the antitumor effect is an important property of the platinum complexes and has led to the suggestion that it is one specific type of interaction of the complex which can take place with the *cis* but not the *trans* that causes tumor regression. One such reaction might involve a *cis* complex linking bidentately to a base in DNA.

Complexes similar to *cis*-platinum but containing other heavy metals are generally inactive, although some, such as the *cis*-palladium complex (table 2), may be of the same

1. Complexes that have antibacterial activity do not necessarily have antitumor activity.
2. Complexes should be neutral.
3. Complexes should contain a pair of cis leaving groups (generally of intermediate lability, eg, Cl or Br, but oxalate and malonate are also effective). This is a necessary but insufficient criterion for activity.
4. The other ligands must play an important role. They should be inert and neutral. Amine systems (as opposed to oxygen or sulfur) are more likely to give active compounds.
5. Other heavy metals (eg, palladium) give complexes inactive or less active than the platinum analog.

FIGURE 1.—Empirical parameters for antitumor activity in platinum complexes.

TABLE 1.—Antitumor effects of pairs of *cis* and *trans* platinum complexes on the PC6 tumor

Complex		LD50 (mg/kg)	ID90 (mg/kg)	TI*
	*cis*	13.0	1.6	8.1
	*trans*	27.0	None	Inactive
	*cis*	56.5	2.6	21.7
	*trans*	18.0	None	Inactive
	*cis*	240	175	13.7
	*trans*	72	None	Inactive

*TI = therapeutic index (LD50/ID90).

order of toxicity. There are some exceptions however; the rhodium complex has significant activity against the PC6 tumor and a therapeutic index against the Walker 256 carcinosarcoma as high as some clinically useful alkylating agents (table 2). Although there is some work in progress on rhodium and other complexes, it seems an area worthy of further investigation.

The Search for More Effective Analogs

A number of approaches have been adopted, some successful, others less so, in the search for new platinum complexes with more favorable properties than *cis*-platinum. In one series the stable ammonia ligand has been substituted by a series of alicyclic amines of increasing

TABLE 2.—Antitumor effects of complexes similar to *cis*-platinum but containing other heavy metals

Complex	PC6 tumor			Walker 256 carcinosarcoma (TI)*
	LD50 (mg/kg)	ID90 (mg/kg)	TI*	
$Pt(NH_3)_2Cl_2$ (*cis*-platinum)	13.0	1.6	8.1	1.6
$Pd(NH_3)_2Cl_2$	28.0	None	Inactive	Inactive
$Rh(NH_3)_3Cl_3$	225	86	2.6	6.1
$Ir(NH_3)_3Cl_3$	1500	None	Inactive	—
$Ru(NH_3)_3Cl_3$	132	None	Inactive	—

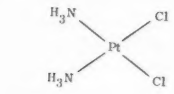
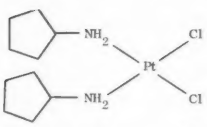
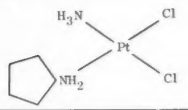
*TI = therapeutic index (LD50/ID90).

TABLE 3.—Antitumor effects of a related series of platinum complexes

Complex	PC6 tumor (TI)*	L1210 leukemia (% ILS)†	Solubility in water (μg/ml)
$(NH_3)_2PtCl_2$ (*cis*-platinum)	8.1	95	2650
$(Cyclopropylamine)_2PtCl_2$	25	70	610
$(Cyclobutylamine)_2PtCl_2$	31	52	87
$(Cyclopentylamine)_2PtCl_2$	200	41	5.6
$(Cyclohexylamine)_2PtCl_2$	> 267	Inactive	1.9
$(Cycloheptylamine)_2PtCl_2$	130	—	0.71
$(Cyclo\text{-}octylamine)_2PtCl_2$	2.9	—	0.29

*TI = therapeutic index (LD50/ID90).
†% ILS = percent increase in lifespan.

TABLE 4.—Antitumor effects of *cis*-platinum derivatives with mixed amines on the PC6 tumor

Complex	PC6 tumor LD50 (mg/kg)	PC6 tumor ID90 (mg/kg)	PC6 tumor TI*
	13.0	1.6	8.1
	480	2.4	200
	11.0	0.5	22

*TI = therapeutic index (LD50/ID90).

ring size (table 3). The therapeutic index measured on the PC6 tumor increased with increasing ring size to an optimal value of about 200, one of the highest indices recorded for this tumor system. However, testing the same samples against L1210 leukemia gave quite opposite results, with antitumor effectiveness decreasing with increasing molecular weight. It now seems likely that the results with the PC6 tumor may be an artifact resulting from the extreme insolubility of the higher members of the series. In experiments against the PC6 tumor, the chemicals were injected ip as ultrasonicates in arachis oil. It is possible that the insoluble members of the series are slowly released and saturate plasma at concentrations sufficient to cause regression of the PC6 tumor but ineffective against L1210 leukemia. Attempts were made to retain the favorable properties of the ammonia ligand but alter the physicochemical properties of the molecule by designing *cis*-platinum derivatives with mixed amines as shown in table 4. However, the general finding was that the mixed amines were no better than the parent complexes.

A more successful approach has involved alteration of the leaving groups as shown in table 5. Substitution of the ammine groups of *cis*-platinum by 1,2-diaminocyclohexane gives a complex with activity similar to that of *cis*-platinum against the PC6 tumor. However, if this is followed by substitution of the *cis*-chloro leaving groups by sulfate, a complex is obtained which is very water-soluble and has a significantly higher therapeutic index against the PC6 tumor.

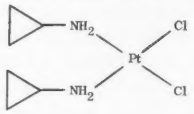
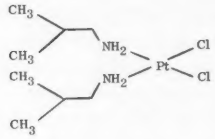
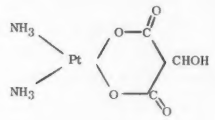
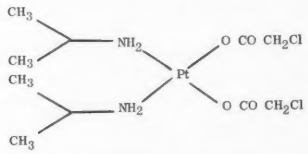
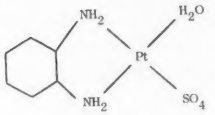
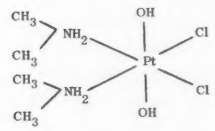
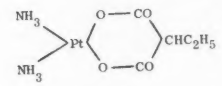
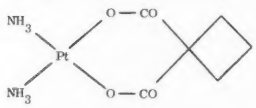
The finding that renal toxicity could be markedly reduced by inducing diuresis in hydrated patients led to the assumption that platinum complexes which are more water-soluble might have less kidney toxicity because of their more rapid excretion. While there is no evidence that platinum complexes which are more water-soluble than *cis*-platinum are, as a rule, less nephrotoxic, one chemical prepared as a result of this research, a *trans*-dihydroxyplatinum(IV) derivative, has proved in tests against the PC6 tumor to be superior to *cis*-platinum and

TABLE 5.—Effects of substitution of the leaving groups and other ligands on the antitumor properties of *cis*-platinum

Complex	PC6 tumor LD50 (mg/kg)	PC6 tumor ID90 (mg/kg)	PC6 tumor TI*
$PtCl_2(NH_3)_2$ (*cis*-platinum)	13.0	1.6	8.1
$PtCl_2$(1,2-diaminocyclohexane)	14.1	2.1	6.9
$Pt(SO_4)(H_2O)$(1,2-diaminocyclohexane)	14.0	0.4	35

*TI = therapeutic index (LD50/ID90).

TABLE 6.—Antitumor effects of selected platinum complexes

Complex	PC6 tumor (TI)*	L1210 leukemia
	24.6	Active
	13.4	Active
	30.6	Active
	Under test	Active
	37.0	Active
	12.0	Active
	13.6	Active
	12.4	Active

*TI = therapeutic index (LD50/ID90).

to be active against a range of experimental tumor systems.

This complex has been subjected to extensive preclinical pharmacology testing and it has been concluded that because of its pharmacokinetic properties it is likely to be less nephrotoxic than *cis*-platinum (3).

Recent Research

The Institute of Cancer Research, in collaboration with the Johnson-Matthey Research Centre, has begun a systematic investigation of eight platinum complexes with the aim of selecting at least two for phase I clinical trials. The structures are shown in table 6; these agents were selected because of their good spectrum of action against the PC6 and other tumors, and their favorable solubility or toxicologic properties compared with *cis*-platinum. The investigation will involve detailed studies on the effects of the complexes on kidney function and their general toxicologic properties. Compounds with low kidney toxicity will be tested further against human tumors of the ovary and testes xenografted to immune-deprived or nude mice. It is also hoped that tests can be devised which will give a quantitative measure of other serious side effects associated with platinum complexes, such as neurotoxicity, particularly ototoxicity, and severe nausea and vomiting.

Some of the complexes referred to above and others containing carboxyphthalato and *bis*-monobromoacetato groups appear to be somewhat better than *cis*-platinum when tested in experimental systems. However, apart from the important fact that they are not necessarily cross-resistant to one another in animal tumor systems, they are all rather similar in their biologic properties. As more is learned about the mechanism of action and the physiologic disposition of this class of chemicals, it is hoped that newer agents with high selectivity will become available.

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Antileukemic (L1210) Activity and Toxicity of *cis*-Dichlorodiammineplatinum(II) Analogs

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SUMMARY

cis-Dichlorodiammineplatinum(II) (_cis_-platinum) and 40 of its analogs were evaluated for antitumor activity in BDF_1 mice implanted ip with 10^6 L1210 leukemia cells. Several of these analogs were also evaluated for their ability to cause elevation in BUN and to produce leukopenia in normal BDF_1 mice. In 11 experiments, _cis_-platinum given on Day 1 or Days 1–9 after implant produced T/C (treated/control) values between 164% and 214% and 157% and 285% respectively. On the basis of single experiments, 13 analogs were judged to be comparable to _cis_-platinum in that they produced T/C values $\geq$ 167% after a single dose or $\geq$ 200% after daily doses for 9 days. These active compounds included various substituted amine derivatives of dichloroplatinum(II), malonatoplatinum(II), aquasulfatoplatinum(II), and chloroacetatoplatinum(II) and derivatives of dihydroxydichloroplatinum(IV). Twelve of these active analogs and _cis_-platinum were evaluated for toxicity at doses ranging from near the optimal therapeutic dose to greater than or equal to the LD50. Only five of the 12 analogs and _cis_-platinum caused an increase in BUN to > 30 mg/100 ml, while eight of the analogs produced leukopenia comparable in incidence and severity to that produced by cis-platinum.

[Cancer Treat Rep 63:1503–1508, 1979]

cis-Dichlorodiammineplatinum(II) (*cis*-platinum) has demonstrated antitumor activity in a number of transplantable animal tumors including L1210 leukemia and sarcoma 180 (1,2), Walker 256 carcinosarcoma and ascitic B16 melanoma,[4] Dunning ascitic leukemia (3), and 7,12-dimethylbenzanthracene (DMBA)-induced mammary tumors (4). *cis*-Platinum has also demonstrated antitumor activity in several human malignancies. However, the dose-limiting toxic effects in patients are nephrotoxicity and severe nausea and vomiting. It is therefore important that analogs of *cis*-platinum be studied to obtain other active but less toxic platinum-containing compounds.

Analogs of *cis*-platinum have been under investigation in animal tumors for several years and some have demonstrated antitumor activity equal to or greater than that of *cis*-platinum (5–8). To date, only two compounds, 1,2-diaminocyclohexylsulfatoplatinum(II) and 1,2-diaminocyclohexylmalonatoplatinum(II), have been administered to a small number of patients (9–11). The relative effectiveness of these agents compared to that of *cis*-platinum has not been evaluated.

Results of screening 40 *cis*-platinum analogs for antitumor activity in mice bearing L1210 leukemia will be presented in this report. The effect on BUN levels and total wbc counts was evaluated for 12 of the 40 compounds.

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[2]Johnson-Matthey Research Corp, Sonning Common, Reading, United Kingdom.

[3]The authors wish to thank Sally Van Camp and Rita Sutliff for typing the manuscript, and Dr. R. Buchanan for his helpful suggestions during these studies.

[4]Handelsman H, Goldsmith N, Broder L, et al. Clinical brochure: *cis*-platinum diamminedichloride. Prepared by the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md, 1976, pp 10–15.

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MATERIALS AND METHODS

Mice.—Adult male and female (C57BL/6 × DBA/2) F_1 (BDF_1) and DBA/2 mice, 6–8 weeks old, were supplied by the Mammalian Genetics and Animal Production Section, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md). Mice were fed Wayne Breeder Blox and water ad libitum.

Leukemia.—L1210 leukemia was maintained in DBA/2 mice by weekly ip transplantation of 10^5 ascites cells.

Drugs.—All platinum analogs and *cis*-platinum were obtained from Matthey Bishop, Inc (Malvern, Pa).

Antitumor testing.—Antileukemia experiments were initiated by implanting BDF_1 mice (all of the same sex in any given experiment) ip with 10^6 L1210 ascites cells. Drug treatment was begun on Day 1 after implant and consisted of either a single ip injection (Day 1 only) or nine daily ip injections (Days 1–9). Leukemia control mice received only saline daily until their death or until Day 9, whichever came first. Experiments were terminated on Day 30 or when no mice remained alive. *cis*-Platinum was included as a positive control in each treatment group. All drug-treated groups consisted of six mice and saline-treated leukemia control groups consisted of ten mice. In all experiments, each *cis*-platinum analog was evaluated at four dose levels using single- and multi-dose schedules. Mice were observed daily and the antileukemic activity of each analog was compared on the basis of %T/C, defined as the median survival time (MST) of the entire drug-treated group divided by the MST of the saline-treated leukemia control group × 100. An analog was considered "active" if it produced a T/C value of ≥ 125% using either the single- or multi-dose treatment schedule. The dose of each analog yielding the greatest %T/C was referred to as the optimum dose (OD). In most instances, the OD was the same as the maximum tolerated dose, defined as the highest dose at which none of the deaths among treated leukemic mice could be attributed to drug toxicity. Deaths attributed to drug toxicity were based on the mortality patterns of the dying mice, ie, deaths occurring prior to the first death among saline-treated leukemic control mice were considered to be drug-induced.

BUN.—Non-tumor-bearing male BDF_1 mice, in groups of five to ten, were bled from the retro-orbital plexus prior to and 4 days after a single ip injection of drug. Serum was collected using Microtainers (Becton-Dickinson, Co, Rutherford, NJ) and BUN was measured using a Centrifichem System 400 (Union Carbide, Co, Rye, NY). BUN values > 30 mg/100 ml were considered indicative of renal toxicity.

Total wbc count.—Non-tumor-bearing male BDF_1 mice, in groups of five to ten, were bled from the retro-orbital plexus prior to and serially at 3, 5, and 7 days after a single ip injection of drug. Total wbc counts were performed on a Coulter Counter Model S (Coulter Electronics, Inc, Hialeah, Fla). A decrease in wbc counts of > 35% of pretreatment values was considered indicative of drug-induced leukopenia.

RESULTS

Antitumor Activity

The therapeutic activity of *cis*-platinum and 40 of its analogs was evaluated in mice bearing L1210 leukemia. All saline-treated leukemic control mice died and their MSTs varied from 6.0 to 7.0 days in the 11 experiments performed. The structure of each analog and its optimal therapeutic activity are summarized in table 1.

Dichloroplatinum(II) analogs.—The parent compound, *cis*-platinum, when given on Day 1 or Days 1–9 after leukemia cell implant, produced T/C values between 164% and 214%, and 157% and 285% respectively. Analogs were described as being comparable in antitumor activity to *cis*-platinum if their T/C values were within the higher range of T/C values achieved with *cis*-platinum. Among the dichloroplatinum(II) analogs evaluated, those containing either isopropylamine (No. 6), isobutylamine (No. 2), or cyclobutylamine (No. 1), substituted at R_1, were comparable in activity to the parent compound. However, none achieved T/C values approaching the maximum achieved by *cis*-platinum.

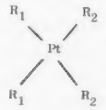
Malonatoplatinum(II) analogs.—Several malonatoplatinum(II) analogs were evaluated and found to have antileukemic activity. Those (compounds 5 and 10) retaining ammine groups at R_1, as in the parent compound, displayed activity on at least one treatment schedule that was comparable to that achieved with *cis*-platinum. Additionally, compound 182, with a diaminocyclohexane group at R_1, had activity comparable to that of *cis*-platinum when given on a multi-dose schedule.

Aquasulfatoplatinum(II) analogs.—All of these analogs were active, although compound 33 had only borderline activity. Included in this group is a 1,2-diaminocyclohexane-containing compound (No. 20) which showed antileukemic activity superior to that of *cis*-platinum in the single experiment performed. Single-dose therapy with this analog caused a T/C value of 217% and multi-dose therapy caused a T/C value of 300%. This was the second analog with this R_1 substitution that had shown good antileukemic activity.

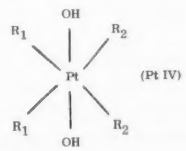
Chloroacetatoplatinum(II) analogs.—Among these analogs studied, two (Nos. 16 and 18) demonstrated antileukemic activity comparable to that of *cis*-platinum. Both analogs had propylamine substitution at R_1, representing both the iso- (No. 16) and normal (No. 18) configurations.

Aminoacidplatinum(II) analogs.—None of the *cis*-platinum analogs with amino acid substitution at R_2 and/or R_1 (compounds 34–37) displayed significant antileu-

TABLE 1.—Therapeutic activity of *cis*-platinum analogs in mice bearing L1210 leukemia*



Compound No.	R_1	R_2	Treatment on Day 1 only			Treatment on Days 1–9		
			OD (mg/kg)	%T/C†	TR‡	OD (mg/kg)	%T/C†	TR‡
			Dichloroplatinum(II) analogs					
cis-Platinum	Ammine	Cl	8	164–214	4	2	157–285	3
3	Methylamine	Cl	16	129	< 2	2	121	—
6	Isopropylamine	Cl	32	171	< 2	8	179	2
11	Cyclopropylamine	Cl	16	157	4	8	164	4
4	Tertiarybutylamine	Cl	64	100	—	32	114	—
2	Isobutylamine	Cl	64	171	4	16	193	16
1	Cyclobutylamine	Cl	32	157	4	16	221	4
			Malonatoplatinum(II) analogs					
40	Ethylenediamine	Malonato	34, 69	129	2	17	164	4
12	Isopentylamine	Malonato	128	129	< 2	8	100	—
13	Cyclopentylamine	Malonato	128	107	—	128	100	—
182	1,2-Diaminocyclohexane	Malonato	32	154	4	16	254	16
5	Amine	2-Hydroxymalonato	64	150	< 2	32	200	2
14	Isopentylamine	2-Hydroxymalonato	64	121	—	32	100	—
15	Cyclopentylamine	2-Hydroxymalonato	128	107	—	128	107	—
10	Ammine	2-Ethylmalonato	128	171	2	64	186	4
			Aquasulfatoplatinum(II) analogs					
19	Isopropylamine	H_2O; SO_4	23	150	8	6, 11	167	2
32	Isobutylamine	H_2O; SO_4	15	150	< 2	7.5	129	< 2
33	*N,N'*-$(ET)_2$-ethylenediamine	H_2O; SO_4	104	129	< 2	26, 52	121	—
20	1,2-Diaminocyclohexane	H_2O; SO_4	11	217	⩾ 8	3	300	⩾ 3
			Chloroacetatoplatinum(II) analogs					
18	*N*-Propylamine	Chloroacetato	12, 24	167	8	6	225	4
16	Isopropylamine	Chloroacetato	32, 64	179	8	16	207	8
29	*N*-Butylamine	Chloroacetato	256	107	—	32	136	4
30	Isobutylamine	Chloroacetato	52	150	4	26	150	4
16A	Cyclopentylamine	Chloroacetato	128	121	—	32, 64	143	2
			Aminoacidplatinum(II) (and derivatives) analogs					
34	Glycinato	Glycinato	223	114	—	112	100	—
35	Alaninato	Alaninato	56	107	—	56	100	—
36	Glycine	Cl	39	129	< 2	39, 78	100	—
37	Alanine	Cl	56	114	—	14–112	100	—
			Other platinum(II) analogs					
21	*N*-Propylamine	Oxalato	59	129	< 2	15, 30	143	2
16B	Isopropylamine	Nitrato	64	171	4	16	193	4
8	Amine	1,1-Cyclobutanedicarboxylato	128	150	2	64	157	2



			Dihydroxydichloroplatinum(IV) analogs					
17	Methylamine	Cl	112	143	4	56	157	2
22	Ethylamine	Cl	104	143	2	13, 26	129	4
42	*N*-Propylamine	Cl	45, 90	129	2	22.5	157	4
9	Isopropylamine	Cl	32	171	4	16	207	2
43	*N*-Butylamine	Cl	104	179	8	26	175	4
44	3-Methoxypropylamine	Cl	69	129	< 2	69	207	4
			Other dihydroxyplatinum(IV) analogs					
28	Isopropylamine	Malonato	256	107	—	64	114	—
26	Isopentylamine	Malonato	256	100	—	16	107	—
38	Glycinato	Glycinato	256	100	—	128	107	—
39	Alaninato	Alaninato	97	100	—	97	100	—

*BDF_1 mice were implanted ip with 10^6 L1210 cells and treatment was begun on Day 1 after implant. All compounds were administered ip.

†Each treatment group (T) contained 6 mice; each control group (C) contained 10 mice.

‡TR = therapeutic ratio: maximum tolerated dose divided by minimum effective dose.

TABLE 2.—Effect of *cis*-platinum analogs on BUN levels and wbc counts in male BDF_1 mice

Compound No.*	OD† (mg/kg)	LD50‡ (mg/kg)	Single-dose effects		
			Test dose (mg/kg)	Incidence of BUN > 30 mg/100 ml	% change in wbc count (Day)
		Dichloroplatinum(II) analogs			
cis-Platinum	8	17.8	17.8	6/6	–18 (5)
			13.4	9/10	–43 (3)
			10	6/9	–34 (4)
			7.5	ND§	–29 (3)
3	16	34 (L)	48	0/5	–68 (3)
			24	0/5	–33 (3)
6	32	40 (L)	48	0/5	–71 (3)
			32	0/5	–21 (5)
			16	0/5	–6 (7)
11	16	40 (L)	32	1/3	+29 (7)
			16	0/5	+51 (3)
4	64	81 (L)	128	0/1¶	–9 (5)
			64	1/2	–8 (3)
2	64	112 (L)	100	ND	–60 (5)
			64	0/5	–39 (3)
			32	0/5	ND
1	32	72 (L)	64	0/5	–62 (5)
			32	0/5	–32 (7)
		Malonatoplatinum(II) and aquasulfatoplatinum(II) analogs			
5	64	102 (L)	128	1/5	–41 (5)
			64	0/2	–44 (3)
182	32	359.2	ND	—	—
10	128	120 (L)	200	1/4	–60 (5)
			100	0/5	–33 (7)
20	11	22.6	22.6	0/10	–67 (5)
			17.0	0/10	–64 (5)
			12.7	0/10	–57 (5)
		Other platinum(II) analogs			
8	128	> 200 (L)	240	1/5	–53 (3)
			160	0/5	–66 (7)
			80	0/5	–32 (5)
9	32	60 (L)	160	ND	–73 (3)
			80	0/5	–75 (3)
			40	0/5	ND
16	32	53.8	53.8	0/10	–37 (3)
			40.4	0/10	–21 (3)
			30.3	0/10	–14 (5)

*All compounds were administered ip.
†Single dose producing highest %T/C.
‡Determined in L1210 leukemic (L) or non-tumor-bearing male BDF_1 mice.
¶4 of 5 mice died before BUN levels were measured.
§ND = not determined.

kemic activity. Three other platinum(II) analogs (Nos. 8, 16B, and 21) with various substitutions at R_1 and R_2 were evaluated. Most significant among the three was compound 16B which had activity comparable to that of the parent compound. This was the fourth analog with isopropylamine substituted at R_1 that was found to be active.

Dihydroxyplatinum(IV) analogs.—Ten analogs containing dihydroxyplatinum(IV) were evaluated. Six of these analogs contained chloride groups at R_2 including three (Nos. 9, 43, and 44) which displayed activity comparable to that of *cis*-platinum. The remaining four dihydroxyplatinum(IV) analogs (Nos. 26, 28, 38, and 39) were inactive. They include analogs with amino acid groups substituted at R_1 and R_2, as well as two analogs with malonato groups substituted at R_2 and either isopropylamine or isopentylamine at R_1.

Toxicity

The effects of several *cis*-platinum analogs on BUN levels and wbc counts in non-tumor-bearing male BDF_1 mice are shown in table 2. The single-dose OD, the LD50 dose, and the test doses are presented to demonstrate the relationship between therapeutic and toxic doses. If the LD50 was determined in L1210 leukemic mice, the test doses generally bracketed the LD50 and/or included the OD. If the LD50 was determined in non-tumor-bearing mice, the test doses began with the LD50 and decreased in increments of 0.75.

Of the 12 analogs tested, three (Nos. 5, 8, and 10) caused an increase in BUN to > 30 mg/100 ml in 20%–25% of the animals treated at doses greater than the LD50 dose. No increase in BUN was observed in these animals at lower doses. Compounds 4 and 11 caused an increase in BUN to > 30 mg/100 ml at doses lower than the LD50. However, in both experiments approximately half of the animals died before BUN measurements could be made. The low incidence of increase in BUN observed with the *cis*-platinum analogs is in contrast to the high incidence (60%–100%) observed with the parent compound.

cis-Platinum and a number of analogs were leukopenic at test doses which were the same as or nearly equal to the OD. Notable exceptions were compounds 4, 6, and 16. Interestingly, compound 11 appeared to cause an increase in the wbc count.

DISCUSSION

Of the 40 *cis*-platinum analogs evaluated, many were found to be active (T/C ≥ 125%) against L1210 leukemia. However, only 13 compounds (Nos. 1, 2, 5, 6, 9, 10, 16, 16B, 18, 20, 43, 44, and 182) were judged, on the basis of a single experiment, to be comparable in activity to *cis*-platinum, ie, these compounds produced T/C values ≥ 167% for single-dose treatment or ≥ 200% for daily dose treatment for 9 days.

From the results obtained with dichloroplatinum(II) compounds substituted in the R_1 position by alkylated amines of varying hydrocarbon chain length, it appears that the isopropylamine and isobutylamine substituents (Nos. 1, 2, and 6) were most active. Compound 2 (R_1 = isobutylamine) showed a high therapeutic ratio on a daily (Days 1–9) dose schedule. In contrast to *cis*-platinum, none of these compounds caused an elevation of BUN. However, each analog produced leukopenia comparable to that of *cis*-platinum at the OD.

The most active analog (No. 20: R_1 = 1,2-diaminocyclohexylamine; R_2 = aquasulfato), which produced T/C values greater than that for *cis*-platinum, caused no increase in BUN but did cause leukopenia. The high antitumor activity of this compound confirms the results previously reported (8), and the compound's potency approaches that of *cis*-platinum.

Of note is the observation that amino acid-substituted *cis*-platinum(II) and *cis*-platinum(IV) analogs were totally ineffective against L1210 leukemia. It is possible that rapid hydrolysis of the carboxyl-platinum bond rendered these compounds inactive.

Studies are ongoing in which the most active *cis*-platinum analogs are being evaluated for toxicity and for antitumor activity in other experimental tumors. Structure-activity data are being evaluated to select candidates for clinical trials.

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Platinum Analogs of Clinical Interest[1]

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SUMMARY

Eight platinum analogs including complexes containing two isomeric forms of 1,2-diaminocyclohexane have received preliminary clinical trials. Dosage and limiting toxicity have been identified for racemic malonato-1,2-diaminocyclohexaneplatinum(II) (PHM) and its *trans*(–)-1,2-diaminocyclohexane isomer (neo-PHM). Dosage and toxicity for racemic sulfato-1,2-diaminocyclohexaneplatinum(II) have been identified and preliminary dosage data for the *trans*(–)-1,2-diaminocyclohexane isomer are presented. Of the four remaining compounds, three have been abandoned due to toxicity or lack of promise as antitumor agents and the fourth is not being pursued actively at the present time. Further trials of PHM, and especially the neo-PHM isomer, in combination with standard agents seem indicated and are underway. The search for platinum analogs of clinical interest may yet identify analogs significantly superior in effectiveness and safety to *cis*-dichlorodiammineplatinum(II).

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Our first clinical trials with platinum coordination complexes began in 1971 with phase I testing of *cis*-dichlorodiammineplatinum(II) (*cis*-platinum). A dose range of 2–2.5 mg/kg, antitumor activity in five of 12 patients treated, and nausea, myelosuppression, and nephrotoxicity were noted (1). Although *cis*-platinum showed antitumor activity (2–4), its narrow therapeutic index in man directed much of our subsequent interest to analogs which might be superior.

MATERIALS AND METHODS

Platinum analogs selected for clinical trial include *cis*-dichloro-bis(cyclopentylamine)platinum(II) (PAD) (5), malonato-1,2-diaminocyclohexaneplatinum(II) (PHM) (6,7), sulfato-1,2-diaminocyclohexaneplatinum(II) (SHP) (7), and bis-(monobromoacetato)-1,2-diaminocyclohexaneplatinum(II) (MBA) (8,9). A platinum uracil blue (PAU) preparation was tested (10). Bis-(monobromoacetato)-*trans*-1,2-diaminocyclooctaneplatinum(II) (BOP) was also tested (11).

Recently, Kidani et al (12) and Speer et al (13) have prepared active isomeric forms of PHM and SHP, selecting the more active *trans*(–) isomers, designated neo-PHM and neo-SHP, for testing.

Figure 1 shows the structures of several platinum analogs. They were synthesized and tested in animals in our chemistry laboratories by R. J. Speer and associates.

Preclinical testing of compounds was based on the L1210 ascites system in BDF_1 mice. Speer et al (13) have evolved a figure of merit (M value) for drugs tested in this model. The formula for M is M = (LD50 × best % ILS)/(ID99.9 × 100), where LD50 is the dose killing 50% of mice, ID99.9 is the dose killing 99.9% of tumor cells (48% increase in lifespan), and best ILS is the increase in lifespan up to 200%. The M value and the survival data in mice for several of these compounds are shown in table 1.

In initial studies of dose and effects, pre-terminal patients were treated without excluding those with prior *cis*-platinum therapy. The analogs were administered alone without other cytotoxic drugs. PHM and neo-PHM were administered by iv drip over 1–2 hours in approximately 250 ml of 5% dextrose in 0.2% saline. SHP and neo-SHP were administered by iv drip over 1–2 hours in 150–250 ml of 5% dextrose in water. Initial SHP doses were given in 1 day. Later doses were given daily for 5 days.

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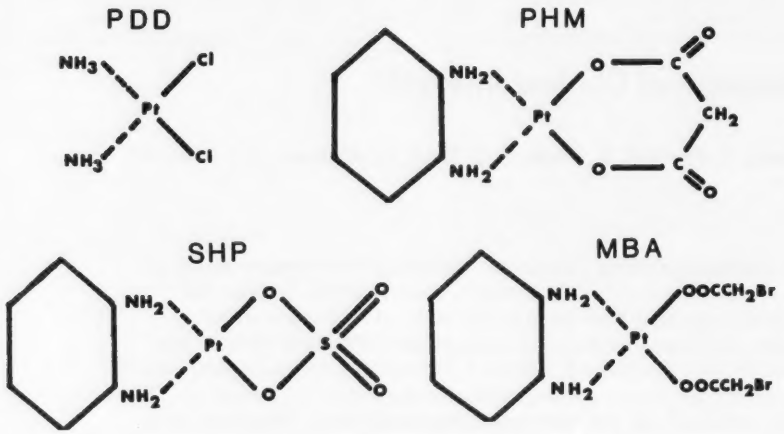

FIGURE 1.—Structures of 4 platinum analogs. PDD = *cis*-platinum.

TABLE 1.—Merit (M) data (*see* text for explanation) for platinum analogs

	LD50 (mg/kg)	ID99.9 (mg/kg)	Best % ILS	M value
cis-Platinum	14	4.2	100	3.3
PAD*	650	93	200	14
PHM	250	22	200	23
Neo-PHM	225–250	10.8–15	200	33–42
SHP	12.5	0.25	200	100
Neo-SHP	8	0.11	200	145
MBA*	27.5	0.27	200	200
BOP*	150	0.75	200	400

*Low solubility required administration as a slurry; LD50 values are probably erroneously high.

Solid tumor responses were rated by percent change in the sum of the products of two perpendicular diameters of measurable lesions. Complete response in patients with leukemia was defined as clearing of blast cells from the marrow and peripheral blood for 30 days. Partial response was defined as clearing of blast cells from the peripheral blood but incomplete marrow response.

RESULTS

PHM and SHP have been the most extensively tested. Results for all compounds are summarized below individually.

PAD.—Patients with various malignancies received PAD. Eight of these patients received the PAD im as a slurry in sesame oil. No therapeutic response or side effects were noted except local irritation. Four patients, including one with acute leukemia and one with multiple myeloma, were given an iv aqueous colloid of PAD in polyvinylpyrrolidone. No adverse effects or therapeutic results were observed in doses up to 10 mg/kg. One patient with liposarcoma also received 15 mg/kg ip in addition to 10 mg/kg iv without adverse reactions.

PAU.—Doses of up to 120 mg/kg iv were given to 12 patients without responses or side effects. No patients with leukemia received this agent. PAU was discontinued following cardiopulmonary arrest in the 13th patient. No autopsy could be obtained.

PHM.—Seventy-eight patients received PHM in phase I studies (14). Twenty-eight had acute or chronic leukemia or related malignancies; 50 had solid tumors. A dose of 16 mg/kg was determined to be suitable. Variations in scheduling included infusion of the total dose over 4 hours and divided doses given daily or weekly to achieve a total dose per course of 16 mg/kg/month. A difference in response has not been noted between these schedules other than slightly less nausea and vomiting when patients received 4 mg/kg/day for 4 consecutive days. The patients received one to seven courses (table 2). The drug was stopped if serious side effects or disease progression occurred. Table 3 summarizes the phase I experience in hematologic and nonhematologic malignancies. Re-

TABLE 2.—Courses of PHM

No. of courses given	Average dose per course (mg/kg)	No. of patients treated
1	14.1	77
2	13.6	24
3	11.2	10
4	12.0	7
5	14.5	4
6	14.0	1
7	14.0	1

TABLE 3.—Antitumor activity of PHM

Tumor type	No. of patients treated	No. of responses
Acute granulocytic leukemia	17	9(2)*
Lung carcinoma	12	1
Colon carcinoma	8	1
Acute lymphocytic leukemia	4	0
Lymphoma	4	1
Chronic granulocytic leukemia	2	1
Chronic lymphocytic leukemia	2	1
Melanoma	2	0
Seminoma	2	1
Thyroid carcinoma	1	0
Miscellaneous carcinomas	12	0

*No. in parentheses = No. of complete remissions.

TABLE 4.—Toxic effects of PHM in 50 patients with solid tumors

Toxic effect	Patients with toxic effects (%)
None	32
Nausea	56
Vomiting	44
Diarrhea	12
Wbc count < 2000/μl	30
Platelet count < 100,000/μl	52
Abdominal cramps	4
Renal toxicity	0
Hepatic toxicity	0

sponses were noted in one of eight patients with colon carcinoma, one of four with lymphoma, one of 12 with carcinoma of the lung, and one of two with seminoma. Minor responses were also noted in one patient each with colon cancer, thyroid cancer, melanoma, and lymphoma. The remaining tumors showed no objective response. Of the 25 leukemia patients, 21 had acute leukemia with 17 acute granulocytic leukemias (AGL) and four acute lymphocytic leukemias. Two of the 17 AGL patients obtained complete remission and seven obtained partial remission. The first patient who achieved complete remission received 12.5 mg/kg iv over 11 hours and 10 mg/kg 6 weeks later. Bone marrow remission was achieved by Day 20. It lasted until Day 60 at which time marrow relapse occurred. Additional PHM at a dose of 14 mg/kg was not effective in obtaining further response. The second patient, who was treated later in the study, achieved a complete remission by Day 27 after receiving PHM at a dose of 15 mg/kg. However, she was not a terminal-phase patient and was, therefore, changed to our standard AGL protocol for long-term care; for this reason, duration of PHM response could not be evaluated. One previously untreated AGL patient experienced nearly complete clearing of blast cells before changing to our standard AGL protocol, following which continued clearing to complete remission occurred. Of the four chronic leukemia patients who received PHM there were two objective responses, one each in chronic granulocytic leukemia and chronic lymphocytic leukemia. Analysis of blast cell response to a single PHM dose reveals that the maximum depression occurred at Day 6. Table 4 lists the side effects and toxicity observed with PHM. The most significant side effects have been gastrointestinal, with varying degrees of nausea and vomiting and, less frequently, severe attacks of diarrhea occurring 2–7 days after PHM. Leukocyte counts of < 2000/μl and platelet counts of < 100,000/μl were noted in 30% and 52% of patients respectively. Bone marrow depression usually resolved within 3 weeks. In three patients who received more than five courses of PHM, peripheral neuropathy was noted. No nephrotoxicity was identified, nor was cardiac, pulmonary, or liver damage observed. The principal limiting toxic effects were neutropenia and thrombocytopenia. Nausea and vomiting were usually manageable and more acceptable than with *cis*-platinum.

SHP.—Twenty nonleukemic patients and two with leukemia received SHP. The first received 0.3 mg/kg followed by 0.2 mg/kg 13 days later. The dose was slowly increased in other patients. One patient received 1.5 mg/kg as a single dose. This patient died 2 days later due to shock which developed immediately after infusion and overwhelming leiomyosarcoma with 90% tumor replacement of the liver. Subsequently, three patients were given 0.3 mg/kg daily for 5 days for a total dose of 1.5 mg/kg. One patient with hypernephroma received seven courses, with several episodes of hypotension following the SHP infusion which were relieved by administration of Levophed and/or prednisolone. However, she was able to continue the medication with no progression of the tumor for 7 months. Sixteen of 20 solid tumor patients receiving SHP lived long enough for the effects on neutrophils, platelets, and serum creatinine to be evaluated (table 5.)

MBA.—Nine patients received MBA, with a slow dose escalation from 0.5 to 2 mg/kg. No effect on the disease was noted. One of the nine patients had a decrease in platelet count to < 100,000/μl and three had decreases to < 50,000/μl. No evidence of nephrotoxicity or hepatotoxicity was noted. One patient who had one fourth of her original dose of 2 mg/kg repeated 1 month later developed a severe allergic and hypotensive reaction. Two other patients received second courses without allergic reactions.

BOP.—Five patients received BOP in doses ranging from 0.25 to 5 mg/kg before trials were discontinued because of nephrotoxicity and myelotoxicity.

TABLE 5.—SHP effects on neutrophils, platelets, and creatinine

Treatment data		Mean values (value before treatment/ value at nadir or peak/Day of nadir)		
SHP dose (mg/kg)	No. of patients treated	Neutrophils/μl	Platelets/μl	Creatinine (mg/dl)
0.2–0.45	5	6291/5695/19	258/103/18	0.9/0.4/17
0.5–0.75	5	4709/3596/19	367/221/14	1.1/1.1/20
0.9–1.2	3	5402/9988*/15	303/205/13	1.2/1.3/14
0.3 × 5 days	3	4456/2537/9	193/28†/14	1.6/0.9/11

*Mean value increased.
†Only 1 value was available for averaging on Day 14.

Neo-PHM.—The desirable dose range for neo-PHM has been determined to be 7.5–8 mg/kg. A maximum of six courses at 7.5 mg/kg and a seventh at 5 mg/kg were given to one patient without nephrotoxicity but with peripheral neuropathy. Morning blood cell counts taken on the first day of therapy revealed mean neutrophil counts of 6879/μl, which declined to a nadir of 971/μl by Day 15, exceeded 2000/μl by Day 20, and were > 7000/μl by Day 29. For platelets, initial mean blood cell counts were 382,000/μl with a nadir of 77,000/μl 12 days later. Levels of platelets were > 100,000/μl at Day 17 and 200,000/μl at Day 22. A patient with chronic granulocytic leukemia who received an infusion of neo-PHM of 7.5 mg/kg responded with a decline in absolute blast cells from 95,000/μl to 105/μl 8 days later. The blast cells, however, gradually returned thereafter.

Neo-SHP.—Neo-SHP was given to four patients beginning at doses of 0.2 mg/kg. One patient received 0.2 mg/kg daily for 4 days and then four monthly courses of 0.2 mg/kg without antitumor effect or toxicity.

DISCUSSION

Eight platinum analogs including two isomeric forms have been evaluated in phase I studies.

PAD was abandoned, with no antitumor effects observed. PAU was abandoned, with no observed benefits or side effects in the first 12 patients. It was not possible to ascertain the role of PAU in the cardiopulmonary arrest in the 13th patient, especially since there was no autopsy.

PHM and particularly the isomer, neo-PHM, have so far shown the most merit for further testing. Of interest was the activity in acute myelogenous leukemia, with two complete remissions and seven partial remissions after a single dose of PHM. None of the leukemia patients had received prior platinum coordination complexes. In contrast, most of the solid tumor patients had received prior *cis*-platinum, although the influence of prior *cis*-platinum treatment on the response to PHM has not been evaluated in this study. Nevertheless, responses were observed in patients with malignancies of the colon, lung, and thyroid, melanoma, seminoma, and lymphoma. If prior *cis*-platinum exposure adversely affects potential responsiveness of patients receiving PHM, this may have slowed our evaluation of therapeutic efficacy to date, but it has not prevented us from determining the suitable dosage or from observing both the side effects of gastrointestinal irritation and the potential toxicity of myelosuppression and occasional peripheral neuropathy. Trials of neo-PHM used alone and in combination are now underway in our center.

SHP has as yet not been extensively studied, although a dose for the racemic mix of about 1.5 mg/kg has been established. It is somewhat myelosuppressive but not nephrotoxic thus far.

MBA studies are not being pursued at present.

BOP has been abandoned at this time. Uncertainty about its M value appears to be due to its inherent insolubility and a false high LD50 figure due to inactivity of nonsolubilized material. In animal experiments it was usually administered as a slurry and its administration to humans was possible only by infusion in large volumes of fluid because of the solubility factor. Although BOP in a colloidal suspension is being re-evaluated in mice, we do not view it as having sufficient promise to return to testing at this time.

In conclusion, we feel that platinum analogs should be further explored and that clinical testing should be carried out especially for neo-PHM and perhaps also for neo-SHP.

The finding of antileukemic activity for PHM may indicate that a spectrum of activity different from that of *cis*-platinum can be found for several such analogs.

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Pharmacokinetics of Non-Protein-Bound Platinum Species Following Administration of *cis*-Dichlorodiammineplatinum(II) [1]

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SUMMARY

The pharmacokinetics of non-protein-bound platinum species derived from *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) was studied under a variety of dosing conditions. Following rapid infusions (15-minute) of *cis*-platinum at 100 mg/m^2, the unbound drug declined in a biphasic mode with a mean terminal half-life of 48 minutes. The mean β-phase half-life after a 6-hour infusion of the same dose of *cis*-platinum was 26 minutes. Urinary excretion of filterable platinum was substantially greater after a 6-hour infusion than after a 15-minute injection. Concomitant administration of mannitol appeared to result in higher peak plasma concentrations and decreased urinary excretion of unbound platinum species but did not alter the terminal half-life. Renal impairment was associated with extremely high plasma levels of filterable platinum but did not affect other pharmacokinetic parameters. Preliminary data on the distribution of *cis*-platinum to ascitic fluid are also presented.

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cis-Dichlorodiammineplatinum(II) (*cis*-platinum) is a chemotherapeutic agent exhibiting antineoplastic activity toward a number of solid tumors. Although the pharmacokinetics of *cis*-platinum has been studied in both animals (1–4) and man (5,6), all such studies have measured only total platinum levels. DeConti et al (5) showed that approximately 90% of the platinum administered as *cis*-platinum was protein-bound within 2 hours after administration, and that the binding was only very slowly reversible. Holdener et al (7) have found in cell culture studies that only the free-circulating platinum species have cytotoxic activity; the protein-bound fraction is inactive. Thus, meaningful pharmacokinetic modeling requires determination of the levels of free-circulating (active) species derived from *cis*-platinum. Analytic methods capable of separating protein-bound platinum from free-circulating platinum have been developed (8,9), and when coupled with X-ray fluorescence or flameless atomic absorption spectroscopy these methods permit the monitoring of the nonbound species.

In a previous paper (10), we reported preliminary results on the pharmacokinetics of unbound platinum species in patients given rapid infusions (15-minute) and 6-hour iv infusions of *cis*-platinum. This report will update our previous data. In addition, we present data on the effects of mannitol on the pharmacokinetics of unbound platinum species as well as the distribution of unbound drug to ascitic fluid and the effects of renal impairment on plasma levels and excretion patterns of filterable platinum species.

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MATERIALS AND METHODS

Patients and Protocol

Patients selected for the study had histologically proven malignancies and a patent lower urinary tract by iv pyelogram; all gave informed consent according to institutional policy. All patients had normal renal function (creatinine clearance $\geq$ 70 ml/minute) except for one patient with ascites from ovarian cancer who had an initial creatinine clearance of 35 ml/minute. Patients were hospitalized, prehydrated with 2000 ml of 5% dextrose in ½ normal saline over 12 hours, and maintained on iv fluids for a minimum of 24 hours after receiving *cis*-platinum.

Patients were randomly assigned to receive one of four possible drug schedules. Three patients received four courses of *cis*-platinum by iv bolus injections (15-minute injection time), and four patients received four courses of *cis*-platinum by bolus injection with 12.5 g of mannitol given by iv push just prior to *cis*-platinum administration and another 12.5 g given immediately following the drug. Another two patients received four courses of *cis*-platinum administered by iv infusion over 6 hours, and three patients received three courses of *cis*-platinum over 6 hours with mannitol administered as above. The dose of *cis*-platinum was 100 mg/m^2. *cis*-Platinum was supplied by the National Cancer Institute (Bethesda, Md) and was administered in sterile, normal saline solution.

Blood samples were collected at 15, 30, 45, 60, and 120 minutes after injection and at the same time points after termination of infusion. Ascitic fluid, when available, was collected at the same time as the blood samples. Total urine was collected for 24 hours, with hourly fractions being collected during the first 6 hours. Although later samples were obtained, the levels of filterable platinum were below the detection limits of the assay (35 ng of platinum/ml) after 2 hours in the case of plasma and after 24 hours in the case of urine.

Method of Analysis

Timed 15-ml blood samples were collected in heparinized evacuated tubes. The plasma obtained by centrifugation of whole blood was placed in Centriflo CF-50-A conical filters (Amicon Corp, Lexington, Mass) and centrifuged at 1000 $\times$ *g* (the maximum recommended acceleration) for 10 minutes. The ultrafiltrate was transparent and contained < 2% of plasma protein as determined by the biuret method. Filterable platinum species were determined as previously reported (9). Two hundred and fifty microliters of ethylenediamine was added to 1 ml of the plasma ultrafiltrate and the mixture was allowed to stand overnight at room temperature. After this time, a 14-mm paper disk impregnated with cation-exchange resin was placed in the vial, and the mixture was ultrasonicated for 45 minutes. The disk was then removed, dried at 100°C for 90 minutes, and stored in a scintillation mini-vial. Platinum appears to be analytically stable indefinitely (> 13 months) on the disk. Hydrochloric acid (250 µl in a 5 M solution) was added to the vials containing the dried resin disks. The vials and contents were ultrasonicated for 15 minutes. The resin disk was then removed from the vial and discarded. A 2-µl aliquot of the hydrochloric acid solution was then introduced into the carbon furnace of a flameless atomic absorption spectrophotometer (Varian Techtron Model 175 B spectrophotometer coupled with a CRA-90 carbon rod atomizer). The platinum 265.95 nm line was monitored. The lamp current was maintained at 10 mÅ. A three-stage heating program of 100°C for 45 seconds, 700°C for 15 seconds, and 2300°C for 1 second was used for analysis. The lamp rate of the program was 600°C/second.

Method of Data Analysis

All plasma data used in this study were fitted to a biexponential equation using a nonlinear regression program based on a simplex algorithm. Justification of the choice of the biexponential model has been presented previously (10).

RESULTS

Mean plasma decay curves for filterable platinum following the termination of rapid (15-minute) and slow (6-hour) infusions of *cis*-platinum with and without mannitol are shown in figures 1 and 2. Following fast infusion there is a biphasic decline in plasma levels of filterable platinum species with a mean initial half-life of 30 minutes and a mean terminal half-life of 48 minutes. In the case of the slow infusion data, the distribution phase after termination of infusion was not as apparent as after rapid infusion. This probably reflects the fact that distribution of non-protein-bound platinum species was largely completed during the period of infusion. The mean terminal half-life was 26 minutes.

For both methods of administration, the use of mannitol produced higher mean plasma levels of filterable platinum species. This finding was consistently seen even within individual patients. Although mannitol affected peak serum levels, there was no effect on the half-life of the unbound drug (table 1).

Urinary excretion of filterable platinum species for rapid and slow infusions is shown in figures 3 and 4 respectively. The fraction of the total dose of *cis*-platinum excreted as filterable platinum in 24 hours is substantially greater after the 6-hour infusion (75%) than after the 15-minute infusion (40%). The use of mannitol resulted in a significant decrease in urinary excretion of filterable platinum species for both infusion schedules. Again, these differences were seen within individual patients receiving courses of *cis*-platinum with and without mannitol.

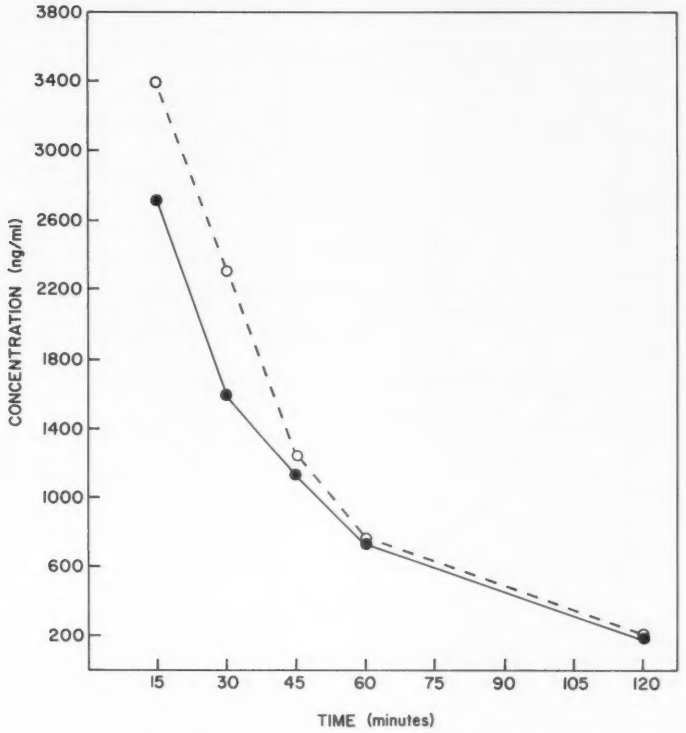

FIGURE 1.—Mean plasma levels of filterable platinum species following administration of *cis*-platinum by rapid (15-min) infusion with (○—○) and without (●—●) mannitol.

In two patients with ascites from ovarian carcinoma, concomitant ascitic fluid and plasma levels of filterable platinum were measured. The data for one patient are shown in figure 5. Ascitic fluid levels of unbound platinum were maximal 30 minutes after administration of *cis*-platinum and persisted at measurable concentrations for up to 24 hours.

Figure 6 shows the plasma levels of filterable platinum in a patient with impaired renal function (creatinine clearance = 35 ml/minute) given a bolus dose of 100 mg/m^2 of *cis*-platinum. This patient experienced severe gastrointestinal toxicity and irreversible renal damage. Despite the high plasma levels, the terminal half-life (53 minutes) was similar to that of patients with normal renal function. The cumulative 24-hour urinary excretion of filterable platinum was 28% of the administered dose compared to an average of 12% for patients with normal renal function who have undergone similar treatment.

DISCUSSION

Following the 15-minute rapid infusion, plasma levels of filterable species decline biexponentially with an initial half-life of 30 minutes and a terminal half-life of 48 minutes. Other studies (5) have reported a biexponential plasma decline of total platinum with an initial half-life of 25–49 minutes and a terminal half-life of 58–73 hours. It therefore appears that the initial plasma decline seen when total platinum is monitored corresponds to the terminal plasma decline of filterable species observed in the present study.

Several clinical methods have been employed to reduce nephrotoxicity associated with *cis*-platinum (11–14). The use of mannitol and slow infusions of *cis*-platinum are among the methods currently being used. The findings reported here show that the half-life of the filterable platinum species in the plasma after termination of the 6-hour infusion is substantially shorter than that observed after the 15-minute rapid infusion (26 versus 48 minutes). Furthermore, mannitol increases the plasma levels of filterable platinum species by 50% in the case of rapid infusion and by 100% in the case of slow infusion, but does not affect the terminal half-life. Measuring total platinum levels in dogs, Cvitkovic et al (4) found that mannitol had no effect on the initial half-life or plasma levels after bolus administration of *cis*-platinum. If their observation is con-

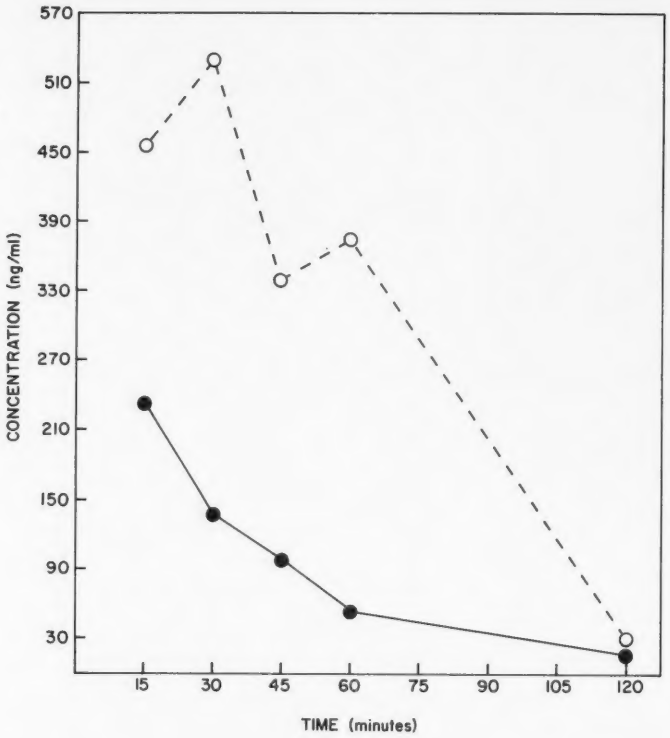

FIGURE 2.—Mean plasma levels of filterable platinum species following 6-hr infusions of *cis*-platinum with (○—○) and without (●—●) mannitol.

TABLE 1.—Summary of filterable platinum disposition after iv administration of *cis*-platinum*,†,‡

Mode of administration	No. of courses	Mean ± SEM 15-min plasma level (μg/ml)	Mean ± SEM β-phase half-life (mins)	Cumulative 24-hr urinary excretion (% ± SEM)
Rapid infusion,§ no mannitol	4	2.73 ± 0.29	48.2 ± 3.2	40.3 ± 4.1
Rapid infusion,§ mannitol¶	4	3.39 ± 1.4	58.7 ± 4.9	16.1 ± 4.1
6-hr infusion, no mannitol	4	0.24 ± 0.02	25.9 ± 2.1	74.9 ± 1.6
6-hr infusion, mannitol¶	3	0.61 ± 0.10	26.0 ± 2.5	19.5 ± 5.1

*Dose, 100 mg/m².

†Patients were administered the drug as described in the text.

‡All of the measurements were made subsequent to completion of administration of drug.

§Administered over 15 mins.

¶12.5 g of mannitol administered prior to and again immediately following administration of *cis*-platinum.

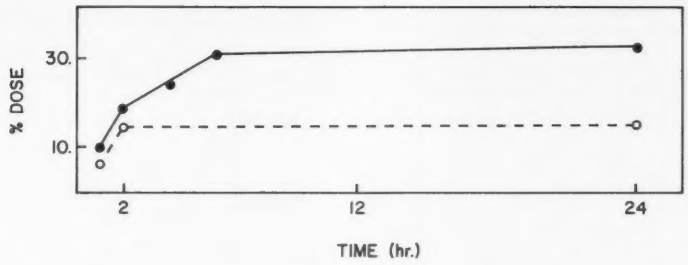

FIGURE 3.—Mean cumulative urinary excretion of filterable platinum species following administration of *cis*-platinum as a 15-min infusion with (○—○) and without (●—●) mannitol.

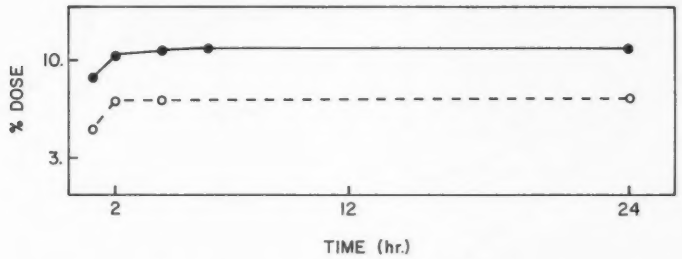

FIGURE 4.—Mean cumulative urinary excretion of filterable platinum species following 6-hr infusions of *cis*-platinum with (○—○) and without (●—●) mannitol.

firmed in our patients, this suggests that mannitol may affect the protein binding of *cis*-platinum.

The urinary excretion of total platinum species has been studied in both man and animals (4–6). From 27% to 70% of an administered dose of *cis*-platinum is excreted in the urine, mostly within the first 4–6 hours after administration. Urinary excretion of filterable platinum depends on the time frame of administration. The mean fraction of the total dose of *cis*-platinum excreted as filterable platinum was significantly greater after 6-hour infusions than after 15-minute infusions (75% versus 40%). The use of mannitol was associated with a substantial reduction in the amount of filterable platinum excreted in the urine in 24 hours. For rapid infusions the mean percent excreted was reduced from 40% in the absence of mannitol to 18% when patients were administered mannitol. After a 6-hour infusion of *cis*-platinum, 75% of the dose was excreted as filterable platinum in 24 hours when patients were not given mannitol, while only 22% was excreted when mannitol was given concomitantly with *cis*-platinum. Measuring urinary excretion of total platinum species in anesthetized dogs, Cvitkovic et al (4) found differences in the urinary concentration of platinum in control, mannitol-treated, and prehydrated animals. However, total urinary recovery of platinum was only slightly lower in the mannitol-treated dogs compared to the prehydrated animals. The differences in the excretion of filterable platinum seen in our patients may again be due to alterations in the binding behavior and reactivity of various platinum species derived from *cis*-platinum.

Filterable platinum levels in ascitic fluid rise quickly to an early peak about 30 minutes after completion of *cis*-platinum administration. The clearance of filterable platinum from the ascites parallels the clearance from plasma, although absolute levels remain higher in ascites compared to plasma. Filterable platinum could be detected in the plasma of the patient with ascites from ovarian cancer for as long as 24 hours after *cis*-platinum administration as compared with detectability for only 2 hours in other patients, suggesting that ascitic fluid may serve as a reservoir for filterable platinum.

In the one patient with impaired renal function, the peak plasma concentration of filterable platinum (43 μg/ml) was higher (four to ten times) than that observed in patients with normal renal function. Furthermore, the cumulative 24-hour excretion in the patient was increased more than twofold relative to patients with normal renal function. The terminal plasma half-life, however, was similar to that observed in patients given *cis*-platinum by a comparable schedule. These data suggest that the clearance of unchanged drug is, at least in part, dependent on renal clearance while the clearance of other fil-

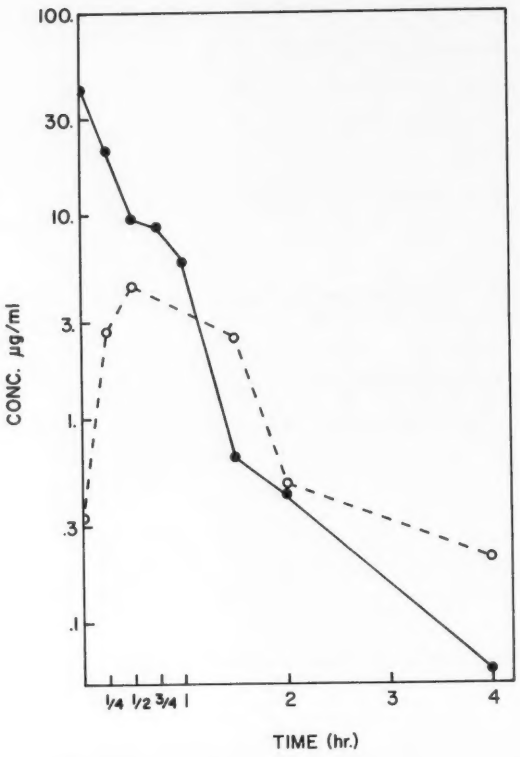

FIGURE 5.—Comparative plasma levels (●—●) and ascitic fluid levels (○—○) of filterable platinum species following a 15-min infusion of *cis*-platinum.

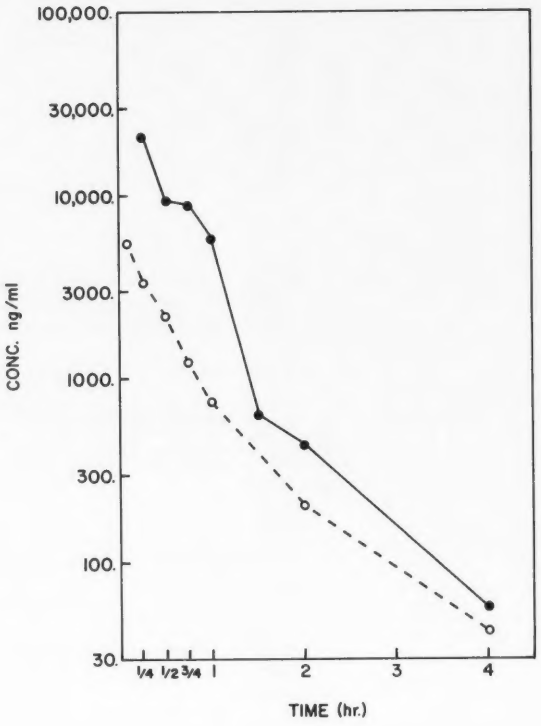

FIGURE 6.—Plasma levels of filterable platinum species in a patient with impaired renal function (●—●) (creatinine clearance = 35 ml/min) given a 15-min infusion of *cis*-platinum compared to mean value for patients with normal renal function (○—○).

terable platinum species is not controlled by renal function.

Further studies are currently underway to determine total platinum levels in the patients reported here and to evaluate the disposition patterns of other platinum species. This will help to more completely elucidate the effects of mannitol and dosing conditions on the binding behavior and reactivity of various platinum species, thus providing greater insight into establishing optimal dosing regimens of *cis*-platinum with less drug-related toxicity.

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Nephrotoxicity of *cis*-Dichlorodiammineplatinum(II)

Irwin H. Krakoff [1,*]

After Rosenberg and his colleagues observed and reported (1) the antitumor properties of inorganic platinum compounds, toxicity studies were done by several groups (2–4) preparatory to clinical trials. Platinum is a heavy metal and it was not surprising that renal toxicity ensued. *cis*-Dichlorodiammineplatinum(II) (*cis*-platinum) and *cis*-tetrachlorodiammineplatinum(IV) exhibited the best antitumor effects among approximately 30 compounds studied. These compounds were also nephrotoxic but exerted little effect on gastrointestinal and gonadal epithelium or on bone marrow. Although it was assumed that the renal toxicity of these compounds was due to platinum as a heavy metal, it is interesting that the *trans*-Pt(II) and -Pt(IV) compounds which did not exhibit antitumor activity also had no effects on the kidney (3).

It was anticipated that renal toxicity might be the dose-limiting toxic effect in man, and this proved to be the case. Renal toxicity was noted to be dose-limiting in an initial clinical experience reported by Speer et al at the 7th International Congress of Chemotherapy in Prague in 1971 (5), and every clinical report at the 2nd International Symposium on Platinum Coordination Complexes in Cancer Chemotherapy held at Oxford University in April 1973 (6) emphasized that problem. Our own initial report (7) was representative of the renal toxicity seen by others; it is summarized in table 1.

The consensus of clinical investigators in 1973 was, as expressed by Hill (6), that "*cis*-platinum(II) diamminedichloride appears to be too good a therapeutic agent to abandon, yet too toxic for general use. . . ."

Gonzalez-Vitale et al (8) studied the histologic appearance of the kidneys in patients who died after having received *cis*-platinum. "Pathologic changes. . .consisted of focal acute tubular necrosis, affecting primarily the distal convoluted tubules and collecting ducts, dilatation of convoluted tubules, and formation of casts."

Several methods have been employed in an attempt to reduce the nephrotoxicity of *cis*-platinum. One of these methods is the use of penicillamine as a chelating agent to bind *cis*-platinum or to complex to renal tubular cell receptors. An early report (9) indicated that penicillamine could prevent renal toxicity in mice with L1210 leukemia and in man. However, this early promise appears not to have been borne out, and penicillamine has not become a useful agent in the prevention of renal toxicity in man.

TABLE 1.—Nephrotoxicity of *cis*-platinum

	cis-Platinum dose (mg/kg)	
	3.0–7.0	1.25–2.5
Total No. of courses	16	19
No. of courses with toxicity degree* of—		
4+	2	0
3+	2	2
2+	10	4
1+	2	3
0	0	10
Serum levels in mg/100 ml (range)		
BUN		
Baseline	14.2 (7–28)	12.2 (6–21)
Peak/Day	38.5 (13–113)/12.8	30/1 (17–56)/8.3
Recovery/Day	17.5 (8–40)/27	15.8 (6–29)/21
Creatinine		
Baseline	0.9 (0.7–1.4)	0.8 (0.6–1.0)
Peak/Day	2.2 (1.2–5.0)/14.5	1.7 (1.3–2.9)/8.0
Recovery/Day	1.2 (0.6–2.5)/26	1.0 (0.6–1.4)/17

*4+ = fatal; 3+ = BUN level rose to more than twice the baseline and remained elevated and creatinine level rose above baseline and remained elevated; 2+ = BUN and creatinine levels rose above baseline but later returned to baseline; 1+ = slight transient rise in BUN level and no rise in creatinine level; 0 = no change in BUN or creatinine level. (Adapted from Lippman et al, ref 7.)

Manipulation of the rate of administration has also been used in an attempt to minimize renal toxicity. Hill et al (10) administered *cis*-platinum by slow infusion at a rate not to exceed 1 mg/kg/hour, and they believed that renal toxicity appeared to occur less frequently than when the drug was given more rapidly. Einhorn and Donohue (11) gave the drug in fractionated doses over a 5-day period; this still produced renal function impairment which was more obvious later rather than in the immediate post-therapy period. When vigorous hydration was added, the incidence of renal damage was said to be less.

Cvitkovic et al (12) demonstrated in studies in dogs that vigorous hydration before, during, and immediately after the administration of *cis*-platinum could protect animals from the renal function impairment produced by *cis*-platinum alone, and that the use of mannitol, an os-

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motic diuretic, facilitated the use of large quantities of fluid. These maneuvers did not influence the serum levels or disappearance times of *cis*-platinum nor did they alter the amount of *cis*-platinum excreted in the urine; they did, however, markedly decrease the urine *concentration* of *cis*-platinum and protected renal function at doses several times higher than those which were tolerated with hydration and mannitol. The effects on leukocyte and platelet counts were not diminished by these maneuvers, suggesting that the antitumor activity, too, would not be abolished. When these studies were extended to man by the same investigators (13), similar results were obtained. Employing a regimen of hydration and mannitol diuresis, doses of 3 mg/kg (approximately 120 mg/m^2) were regularly tolerated without renal toxicity, an increase of about threefold in the tolerated dose. Patients so treated continued to exhibit leukopenia and thrombocytopenia, demonstrating that the effect of *cis*-platinum on dividing cell populations was not abolished. More importantly, the antitumor effect was preserved, with an overall response rate of 42% in the initial series reported, a group of heavily pretreated patients with various tumor types. Of considerable interest was that six patients with germ cell tumors of the testis, known to be resistant to conventional doses of *cis*-platinum (1 mg/kg), exhibited responses to the larger dose (3 mg/kg) used in this study. This suggested very strongly that the technique employed improved the therapeutic index of *cis*-platinum.

Furosemide has also been used (14) in attempts to modify the renal toxicity of *cis*-platinum. However, the reported studies have utilized much lower doses of *cis*-platinum than those used in the study by Hayes et al (13), and it is not clear whether furosemide is as useful as mannitol for that purpose. Furthermore, the direct tubular effects of furosemide suggest a theoretic basis for rejecting it in favor of mannitol, the action of which is osmotic.

Determination of the most effective regimen is difficult because of the lack of comparative studies. It is clear that each of the regimens proposed has some utility in preventing nephrotoxicity. The administration of *cis*-platinum slowly or in fractionated doses produces less nephrotoxicity than does administration by single large-dose bolus, but it is not certain whether or not that schedule sacrifices some antitumor activity.

Each of the maneuvers used to protect the kidneys has shown some activity, but again, the relative merits of these maneuvers have not been assessed in a way which permits comparisons to be made. Thus, the choice of regimen is largely arbitrary. In my opinion, however, the weight of evidence favors the administration of *cis*-platinum in a large single dose with renal protection by vigorous hydration and an osmotic diuretic as proposed by Hayes et al (13).

A further problem in the use of *cis*-platinum may arise from its use with other nephrotoxic agents. Gentamicin has been shown (15) to produce acute tubular necrosis, and as its use in treating infections has proliferated, its nephrotoxic potential has become well-recognized. It is frequently necessary to use antibiotics in patients with advanced neoplastic disease, and it is not surprising that the coincident use of gentamicin and *cis*-platinum has been reported (16) to produce acute renal failure. The histologic appearance of the kidneys in patients treated with gentamicin and *cis*-platinum is similar and the combination produces a similar but more marked picture of tubular damage. This has led to the warning (16) that the two agents should be used together only with great caution, if at all.

Preliminary data in rats, obtained in our laboratories,[2] indicate that the nephrotoxic activity of gentamicin can be prevented by the use of isosorbide, an orally administered osmotic diuretic. It thus seems likely, though not yet firmly established, that isosorbide may prevent the nephrotoxic effects of both gentamicin and *cis*-platinum.

It is encouraging to note that the frustrations expressed at Oxford in 1973 have yielded to enthusiasm regarding this compound, as demonstrated by its widespread use. *cis*-Platinum is still nephrotoxic but the effects are manageable. It has been demonstrated in animals (17) that some of the newer derivatives of *cis*-platinum are less toxic to the kidneys and we can hope that some of these agents will soon find their way into clinical trial.

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Toxic Effects of *cis*-Dichlorodiammineplatinum(II) in Man

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SUMMARY

Administration of *cis*-dichlorodiammineplatinum(II) may be associated with a number of serious side effects, including nephrotoxicity, gastrointestinal side effects (nausea, vomiting, and diarrhea), myelosuppression, and occasional transient elevations in liver function tests. In addition, ototoxicity (tinnitus and hearing loss), anaphylactic reactions, peripheral neuropathies, and hypomagnesemia with resulting tetany may also be encountered. The toxic potential of this new agent necessitates careful clinical monitoring during treatment.

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The importance of *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) in the field of cancer treatment is becoming increasingly recognized. Most of the toxic effects that may be encountered with this new agent were noted in the early phase I trials, and nephrotoxicity was commonly identified as the dose-limiting factor (1). This review will focus on other aspects of *cis*-platinum toxicity based on qualitative and quantitative information obtained in phase II–III trials.

Gastrointestinal Toxicity

Gastrointestinal toxicity in the form of nausea and vomiting has been reported in most studies using fixed dosage regimens of *cis*-platinum (2–19). The incidence of nausea ranges from 50% to 100%, and the incidence of vomiting ranges from 17% to 100%. Nausea and vomiting usually begin 1–6 hours after *cis*-platinum administration (6, 7,20–22). They usually do not last > 24 hours (7,8), but anorexia and nausea may persist for up to a week (6, 7,13,20,22). In a number of studies, patient refusal to receive further *cis*-platinum therapy has been related to severe drug-induced nausea and vomiting (3,4,23). These effects have also been considered dose-limiting by some investigators (11).

The impact of the various doses and schedules on the incidence and severity of these toxic manifestations is difficult to ascertain. Some investigators believe gastrointestinal toxicity is somewhat dose-dependent (21), whereas others believe severe nausea and vomiting can be seen at any dose level (4). The mode of administration may make a difference. Early studies at the M. D. Anderson Hospital using 20 mg/m^2 daily of *cis*-platinum as a continuous iv infusion for 5 days showed a 17% incidence of vomiting (18). However, this interesting observation must be tempered by the fact that all of these patients were hospitalized and well-hydrated. In addition to schedule alterations, iv hydration before and after *cis*-platinum seems to lessen the severity of drug-induced nausea and vomiting (7,9,14). A number of antiemetics have been used to prevent or treat nausea and vomiting. For the most part, none have been consistently helpful (4,7). A new experimental antiemetic, Nabilone, has been reported to have salutary effects against *cis*-platinum-induced gastrointestinal upset (24). Beneficial effects have also been reported with metoclopramide (25). Further double-blind trials should be designed to confirm these early results with both Nabilone and metoclopramide. Clearly, *cis*-platinum-induced nausea and vomiting is a major problem, and more effective means to combat these bothersome side effects are needed.

Diarrhea has occasionally been encountered (11,13,22). No stomatitis has been reported with *cis*-platinum administration (23). Pancreatitis was described in canine tox-

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icology studies (26) but has not been noted in man. Elevations in SGOT and SGPT levels have been noted (2,5,13,23) but all have been transient and mild.

Myelosuppression

Even though *cis*-platinum has generally been regarded as nonmyelosuppressive, several investigators indicate hematologic toxicity may be dose-limiting (10,12). The drug affects all three blood elements.

Leukopenia.—Large variations in the incidence of leukopenia have characterized the phase II trials with *cis*-platinum (table 1). The variety of doses and schedules, the heterogeneity in patient selection, and the different definitions of leukopenia may account for these variations in different trials. The incidence of leukopenia according to the definitions in table 1 ranges from 0 to 50%; wbc counts of $< 1500/mm^3$ were seen in 3% of the patients. The degree of leukopenia seems to be dose-related (7,21) and may be more common with the weekly schedule of drug administration (10,12). Leukopenia may be cumulative but is usually reversible (5,9,21). It is generally more severe in patients who have been extensively pretreated with many other cytotoxic drugs, particularly alkylating agents, and in patients who have had extensive prior radiotherapy (3,7). The day of wbc count nadir varies with the schedule of drug administration. Onset of leukopenia has occurred between Days 6 and 26 (21), and the time to recovery has ranged from Days 21 to 45 (12,21).

Thrombocytopenia.—Thrombocytopenia has also been reported as a dose-limiting toxic effect of *cis*-platinum (12). Again, there are variations among the different trials due to various definitions of thrombocytopenia, different prior treatments, and different doses and schedules. In the various phase II trials reviewed, the incidence of thrombocytopenia (platelet count $< 100,000/mm^3$) ranges from 2% to 50% (2,4,6–15,17–19). Overall, 6% of patients had nadir platelet counts of $< 50,000/mm^3$ (2,4,6–15,17–19). Thrombocytopenia is dose-related and may be cumulative (21), but is usually reversible (9); again, it is generally more severe in patients with a history of prior drug or radiation therapy (2,3,7). The onset of thrombocytopenia is from Days 10 to 26 (21), and the time of recovery ranges from Days 28 to 45 (12,21).

Anemia.—Anemia is a very difficult toxic effect to assess because it may have a complex etiology in oncology patients. A number of investigators feel that *cis*-platinum has a particular effect on the erythron (12,20). The incidence of *cis*-platinum-induced anemia (hemoglobin drop of > 2.0 g/100 ml) ranges from 9% to 40% (2,4,6,7,9, 12,14,19). There is no clear relationship between the dose or schedule of *cis*-platinum administration and the severity of anemia. Some clinicians have speculated that, since erythropoietin is produced by the glomerular tuft of the kidney, the nephrotoxic *cis*-platinum harms the erythropoietin-producing cells, resulting in a normocytic anemia (20). Basophilic stippling of erythrocytes, secondary to *cis*-platinum administration, has never been described. This suggests a mechanism of action for *cis*-platinum toxicity that is different from the ribosomal injury induced by lead. *cis*-Platinum is thought to exert a toxic effect on DNA rather than producing enzyme inhibition or ribosomal injury (12,20).

In summary, *cis*-platinum can cause suppression of all three blood elements. Although it is still unclear which schedule or mode of administration causes the most myelotoxicity, it is clear that blood component support may be required when treating patients with *cis*-platinum.

Neurotoxicity

Ototoxicity.—Ototoxicity secondary to *cis*-platinum consists of tinnitus (13,21,23,27,28) with or without clinical loss of hearing (13,20,21,27,28). In some instances it has been dose-limiting (23). Ototoxicity has been inconsistently studied in clinical trials because definitions of significant hearing changes are variable, patients are ill and unable to cooperate for audiograms, and *cis*-platinum dose schedules have been variable. Within these limitations, the incidence of tinnitus in phase II clinical trials with *cis*-platinum has been 9% (range, 0–33%) (2–7,11–14,18). It is unclear whether tinnitus is dose-related, but is usually reversible (12,14,28). Clinical hearing loss resulting in patient complaints of decreased hearing acuity has been uncommon in clinical trials, with an overall incidence of 6% in compiled series (2–8,11–14,18,19). Because it is uncommon, there is no information on its reversibility. Clinical deafness does seem to be related to the dose of *cis*-platinum administered (5,29). The incidence of audiogram abnormalities, which are more common than clinical hearing loss, varies from 0 to 90% with an overall incidence of 24% (3–6,11,19). These audiogram abnormalities, which usually appear within 4 days after drug administration, consist of at least a 15-decibel loss in pure-tone threshold and tend to be bilateral (5,29). There is a rising incidence of audiogram abnormalities with increasing doses of *cis*-platinum; the damage seems to be cumulative and is not reversible. The audiogram abnormalities are most common in the 4000- and 8000-Hz frequencies which are above the speech range of 1000–4000 Hz (5,20,23,28,29). At the present time it is unclear if patients with a baseline hearing deficit or older patients are more prone to *cis*-platinum-induced audiogram changes (5,21,29). It is of note that a number of clinical trials utilize concomitant furosemide administration to induce diuresis. Furosemide has been indicted as an ototoxic agent (30) and its use with *cis*-platinum could theoretically add to the ototoxicity of *cis*-platinum. Aminoglycoside antibiotics could also theoretically contribute to the ototoxicity of *cis*-platinum, but this potential syner-

TABLE 1.—Leukopenia noted in studies with *cis*-platinum

Dose and schedule	No. of patients	Definition of leukopenia (wbcs/mm³)	% of patients with leukopenia	Ref No.
1.25–1.6 mg/kg, q3wks	24*	1900–4200	17	4
3–5 mg/kg, q4–6wks	60*	< 2000	22	5
50 mg/m², q5wks	31*	< 3000	0	6
30 mg/m²/day × 3, q3wks	11*	< 1300	6	7
30 mg/m², q2–3wks	18*			
50–100 mg/m², q3wks	20*	< 1000	0	8
50 mg/m², q3wks	19*	1000–2000	37	9
40 mg/m², weekly	19*	< 1700†	42	10
60 mg/m², weekly × 6–8	24*	< 1000	8	12
2–4 mg/kg, q4wks	78	< 1000	9	13
		< 2000	22	
		< 4000	49	
2.5–4.5 mg/kg, q3–4wks	26*	< 2000	0	14
100 mg/m², q3wks	25*	2400	4	15
70 mg/m², q3–4wks	24*	< 3000	50	2

*Prior chemotherapy and/or radiotherapy.
†Polymorphonuclear leukocytes.

gism has not yet been observed clinically. Repeated audiograms with each dose of *cis*-platinum administered are probably of limited use since most of the pure-tone threshold loss is in the high-frequency and not in the speech-frequency range. For monitoring purposes, baseline audiograms are recommended. If *clinical* hearing changes become apparent or are reported by a patient, repeat audiograms should be obtained before the next *cis*-platinum dose. If there are significant changes in the speech range, a clinical decision must be made regarding discontinuation of the drug. The pathology of the ototoxicity in animal models seems to be death of hair cells of the organ of Corti (31,32). The pathology of the hearing loss in man has not been studied.

Other neurotoxicity.—A number of investigators have reported instances of neurotoxicity presumably secondary to *cis*-platinum administration (7,9,33). The types of neurotoxicity described have included well-documented peripheral neuropathies, which are usually sensory in nature but occasionally also include sensory plus motor (especially gait) difficulties (9,33,34). Other forms of neurotoxicity, which are less well-documented, include seizures, loss of taste, memory loss, intention tremor, and postural hypotension (7,9,33,34). Sural nerve biopsies from patients who have exhibited paresthesia of upper and lower extremities with gait disturbances have shown light and electron microscopic features consistent with a segmental demyelination pattern of peripheral nerve injury (34). Additional pathologic studies are needed to corroborate these findings. A clinical dilemma may arise when the tumor is responding to *cis*-platinum but drug-induced neurotoxicity develops. The few patients who have had the treatment stopped because of neurotoxicity have had stabilization of their neurologic picture (33,34). Additional systematic studies of this toxicity are needed before a recommendation can be made regarding continued drug administration in the setting of *cis*-platinum-induced neurotoxicity.

Allergic Reactions

Allergic reactions to *cis*-platinum have included skin rash (5) and anaphylactic reactions (5,10,22,35,36). The occurrence of these reactions should not be surprising since industrial exposure to dusts of complex salts of platinum can cause asthma and immediate and delayed hypersensitivity reactions in man (37,38). Skin rashes are rarely seen but can be urticarial or nonspecific maculopapular rashes. Serious anaphylactic reactions were originally reported by Khan and colleagues (22). Since their report, a number of other anaphylactic reactions have been observed (5,10,35,36).

In the Medicine Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, there have been a total of four serious anaphylactic reactions in 107 patients who have received the drug (total of 267 courses) over the past 3 years. These reactions consisted of nearly immediate flushing, facial edema, wheezing, tachycardia, and hypotension. All patients in the Medicine Branch series, as well as those reported in

the literature, had received prior *cis*-platinum therapy and a number of them had atopic personal or family histories. All reactions have been controlled with prompt iv administration of antihistamines, epinephrine, and/or glucocorticoids. There are a few reports in the literature describing patients who, in spite of severe anaphylaxis from *cis*-platinum, were re-treated safely with *cis*-platinum with concomitant use of antihistamines and/or corticosteroids (5,10,36). The number of patients pretreated in this manner is too small to make firm recommendations as to the safety of this management in preventing further anaphylactic episodes. It is important, however, to be aware of the potential for anaphylactic reactions in any patient who receives *cis*-platinum. The patient should be watched closely while the drug is administered and resuscitation equipment and drugs should be readily available. The possibility that non-cross-reactive analogs of *cis*-platinum will have a role in circumventing reactions in patients who are allergic to *cis*-platinum is an area deserving future exploration (22,37).

Miscellaneous Toxic Effects

Cardiotoxicity.—Myocarditis has been documented in monkeys receiving *cis*-platinum (26). However, cardiac toxicity of *cis*-platinum has been very rare in man. There has been a report of one patient with ST-T wave changes and congestive heart failure which was thought to be secondary to *cis*-platinum (21). Appearance of left bundle branch block has been reported in another patient (39).

Hypomagnesemia.—A potentially serious toxic effect of *cis*-platinum is drug-induced hypomagnesemia. Recently, two patients receiving *cis*-platinum therapy at the National Cancer Institute had muscle twitching, tetany, and serum magnesium concentrations of 0.6 and 0.36 mEq/liter respectively (normal = 1.4 mEq/liter). Calcium levels were normal in these patients. These life-threatening cases of tetany, both of which were corrected by magnesium replacement, prompted a retrospective review by Schilsky and Anderson (40). Of 37 patients who had received *cis*-platinum alone in a phase II trial, 21 (57%) developed hypomagnesemia. There was no clear relationship between the hypomagnesemia and the amount of *cis*-platinum administered. A prospective study by the same investigators has shown the hypomagnesemia to be most likely due to inappropriate urinary magnesium losses, probably secondary to a *cis*-platinum-induced renal tubular defect in magnesium conservation (40). *cis*-Platinum administration demands careful followup of serum magnesium levels and attention to patient complaints of muscle irritability. Instances of hypocalcemia have also been recorded in patients receiving *cis*-platinum.

In summary, *cis*-platinum administration leads to a number of toxic effects common to many anticancer compounds, including gastrointestinal side effects (nausea, vomiting, diarrhea, and occasional liver function test abnormalities) and myelosuppression. *cis*-Platinum is also nephrotoxic, which has been a prominent dose-limiting characteristic of the drug. Other less common, but important, side effects include ototoxicity (tinnitus and hearing loss), anaphylactic reactions, peripheral neuropathies, and hypomagnesemia, eventually with tetany. The occasional severity of these toxic effects points to the need for close monitoring of *cis*-platinum treatments.

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cis-Dichlorodiammineplatinum(II)-Based Chemotherapy as Initial Treatment of Advanced Head and Neck Cancer [1]

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SUMMARY

During the past 2–3 years, a total of 73 patients with advanced head and neck cancer (mostly stage IV) were treated with *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) as initial therapy, either alone or in combination with other agents. After chemotherapy, the patients received surgery and/or radiation therapy in standard fashion. Major degrees of tumor regression were seen in 40%–71% of the patients. *cis*-Platinum and bleomycin given by infusion gave the highest response rate (71%). The addition of high-dose methotrexate to *cis*-platinum plus bleomycin produced an unacceptable level of toxicity. Similarly, a four-drug regimen in which low-dose methotrexate and vinblastine were added to *cis*-platinum and bleomycin resulted in increased toxicity without additional therapeutic effect. Initial chemotherapy does not compromise subsequent surgery or increase either the immediate complications of surgery or the acute toxicity of radiation therapy. It seems unlikely that initial chemotherapy has altered the grim prognosis of an advanced, inoperable presentation. The effect of initial chemotherapy on advanced, operable disease will have to be assessed by suitable controlled trials.

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The need for new active antineoplastic agents for the treatment of human cancer is well-illustrated by epidermoid carcinoma of the head and neck. Therapy with both single agents and various drug combinations has generally produced short remissions of marginal usefulness (1). After the discovery of the antitumor activity of platinum complexes by Rosenberg and co-workers, clinical trials quickly demonstrated that *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) was active against a range of human malignancies including head and neck cancer (2,3), but only at the expense of rather severe nephrotoxicity that seriously limited its clinical use. Hayes et al (4) then showed that a vigorous hydration program with a mannitol-induced diuresis accompanying administration of *cis*-platinum was highly protective of renal function; this work served to quicken interest in *cis*-platinum once more. Re-evaluation of the drug in head and neck cancer showed impressive activity with tolerable toxicity (5). Because of the magnitude of many of these responses, our major thrust was then directed toward the previously untreated patient with advanced disease. We felt that a drug which showed a high order of activity in heavily pretreated patients might be substantially more active if used as initial therapy. We describe here our results to date with this approach.

MATERIALS AND METHODS

All patients had histologically confirmed epidermoid or anaplastic carcinoma from a primary site in the head and neck or from a cervical lymph node if no primary site was evident. All patients had measurable disease and normal renal function. Details of the pretreatment evaluation have been described previously (6).

Four regimens were studied in sequence (fig 1). Chemotherapy was administered as initial treatment, and

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Regimen A
cis-Platinum, 120 mg/m^2 iv, Day 1
Bleomycin, 10 mg/m^2 iv push, Day 3
Bleomycin, 10 mg/m^2 continuous iv infusion, Days 3-10

Regimen B
Methotrexate, 50 mg/m^2 iv push, Day 1 then methotrexate, 1500 mg/m^2 continuous iv infusion for 36 hrs

followed by

Leucovorin, 25 mg/m^2 iv push and 200 mg/m^2 iv in 12 hrs
Leucovorin, 25 mg/m^2 im q6hrs x 6 doses

followed by

Regimen A, Days 4-14

Regimen C
Methotrexate, 40 mg/m^2
Vinblastine, 7 mg/m^2 } iv, Day 1
Bleomycin, 20 mg/m^2
Bleomycin, 20 mg/m^2 continuous iv infusion, Days 1-4 inclusive
cis-Platinum, 100 mg/m^2, Day 5

Regimen D
cis-Platinum, 120 mg/m^2 iv

FIGURE 1.—*cis*-Platinum regimens.

was followed by either radiation therapy, surgery, or a sequential combination of the two. During our first protocol (regimen A in fig 1) only inoperable patients with massive disease were treated; hence, radiation therapy was used uniformly in patients receiving subsequent treatment. In the programs which followed, however, patients with operable (although advanced) lesions were increasingly included; in such cases surgery was often followed by postoperative radiation (table 1). Radiation therapy was delivered by standard techniques which have been previously outlined (6). Following completion of surgery and/or radiation therapy, the patient was followed closely for signs of recurrence. We had originally (6) intended that all patients completing primary therapy would receive a maintenance regimen including cyclophosphamide, Adriamycin, and methotrexate. We soon found, however, that the delivery of such a regimen was impractical in patients just completing rigorous combination therapy. The vast majority of the patients, therefore, received no further chemotherapy in the absence of relapse.

Response categories are as previously defined (6). For regimens A, B, and C, responses were assessed approximately 3 weeks from the start of therapy. For regimen D, patients showing response at the 3-week point were generally given further doses of *cis*-platinum until maximum response was achieved; the maximum response was then taken as the response of the patient.

RESULTS

Regimen A

A detailed description of the characteristics of this regimen, including responses and toxicity, has already been published (6) and is summarized in table 2. Only two of the 21 previously untreated patients started in this regimen are currently alive without evidence of disease at 24 and 25 months respectively from the start of treatment. A third patient is alive with disease at 23 months. Despite the lack of a control group, it seems clear that the chemotherapy exerted no significant effect in increasing the frequency of long-term survival in these patients with very advanced disease.

Regimen B

At the time our work with regimen A was in progress, Goldberg et al (7) demonstrated that a 36-hour infusion of high-dose methotrexate followed by leucovorin rescue showed suggestive evidence of activity in previously untreated head and neck cancer patients, while also showing an apparent lack of significant side effects. We therefore combined these two regimens in a fashion which would, we hoped, produce therapeutic enhancement without increased toxicity and thus might qualify the regimen for suitable preoperative adjuvant therapy. We chose this particular dose and schedule of methotrexate since Goldberg et al had shown that the very high peak blood levels of methotrexate obtained with other regimens were avoided. We thus felt that an unacceptable level of kidney damage which might otherwise follow the administration of two nephrotoxins in such close temporal proximity could be averted.

After treating a total of 11 patients, nine of whom had had no prior therapy, we abandoned the use of this program, for its toxicity was formidable (table 2). One of the three patients whose peak creatinine levels were > 2 mg/dl died in renal failure, having also sustained a myocardial infarct, pneumonia secondary to drug-induced leukopenia, and gastrointestinal bleeding at a time of severe thrombocytopenia. Profound leukopenia was infrequent, with only one patient developing a wbc count of $< 2000/mm^3$; only one episode of infection secondary to leukopenia occurred. Seven of the patients developed a platelet count of $< 100{,}000/mm^3$; in five of these patients the nadir was $< 50{,}000/mm^3$, although only one episode of clinically significant bleeding occurred. Both wbc and platelet nadirs occurred at a mean of 10 days following initiation of

TABLE 1.—Patient characteristics and sequence of therapies (staging according to the classification of the American Joint Committee [1977])

	Regimen				
	A	B	C	D	Totals
Total No. of patients	21	9	21	22	73
Males	13	5	17	16	51
Females	8	4	4	6	22
Primary tumor					
Oral cavity	3	1	6	5	15
Nasopharynx	3	1	1	2	7
Oropharynx	10	5	7	8	30
Hypopharynx	1	1	3	3	8
Larynx	1	1	2	1	5
Skin	1	0	0	1	2
Paranasal sinuses	0	0	1	1	2
Unknown primary	2	0	1	1	4
Stage III	0	2	6	3	11
Stage IV	21	7	15	19	62
Positive nodes	16	7	18	16	57
Negative nodes	5	2	3	6	16
Chemotherapy only	1*	3†	2‡	0§	6
Chemotherapy-radiation therapy	19	5	6	12§	42
Chemotherapy-surgery	0	1	4	0§	6
Chemotherapy-surgery-radiation therapy	0	0	9	10§	19
Chemotherapy-radiation therapy-surgery	1	0	0	0§	1

*1 death from drug toxicity.
†1 death from disease; 1 death from drug toxicity; 1 unevaluable patient (*see* text).
‡1 death from myocardial infarct after completing chemotherapy; 1 patient lost to followup.
§Anticipated therapy.

methotrexate therapy. Eight of the patients developed mucositis; in six patients this was severe enough to interfere with oral intake. In one patient the mucositis was clearly related to methotrexate alone, since bleomycin administration had not yet begun. In another patient, the bleomycin infusion had to be discontinued after 4 days because of florid oral ulceration. In another four patients the mucositis became severe after conclusion of the bleomycin infusion but was not sufficiently developed during the infusion to warrant its discontinuation. Two patients developed maculopapular rashes during therapy. One patient had the first evidence of a rash on Day 1 of the bleomycin infusion which was given as usual without untoward consequences, except for transient worsening of the rash. The other patient developed a rash on Day 4 of the bleomycin, which was continued to completion; the rash became dramatically worse with partial exfoliation and then subsided several days following termination of the infusion.

After treatment with chemotherapy, three of the nine previously untreated patients received no further therapy; one patient died of disease on Day 13 of the protocol, one died of drug toxicity on Day 15, and one, whose chemotherapy had to be interrupted before completion because of systemic arterial embolization, was too debilitated to continue with the therapy. Of the six patients remaining, one was treated with definitive surgery and the other five were treated with full doses of therapeutic irradiation (one of these five had resectable disease but refused surgery). At the end of surgery or radiation therapy, four of the patients had been rendered disease-free by combined modality treatment; the remaining patient had a minor response to radiation and subsequently died of metastatic disease. One of the four patients with no evidence of residual cancer died without evidence of disease 3½ months from the start of treatment of progressive pulmonary infection due to uncontrolled aspiration. Two patients relapsed with recurrent disease within the radiation field 4 and 6 months from the start of treatment. Another patient (the only one of the group who did not receive radiation) developed a second primary cancer of the oral cavity 9 months from the start of treatment; this was treated with peroral excision. Two patients continue disease-free 17 and 18 months from the start of therapy. A third patient, whose prior treatment had included a neck node biopsy and a hemithyroidectomy for anaplastic carcinoma not of thyroid origin, was treated with chemotherapy for persistent measurable disease followed by radiation therapy; she is alive without disease at 15 months.

TABLE 2.—Results of initial chemotherapy

	Regimen				
	A	B	C	D	Totals
Total No. of patients	21	9	21	22	73
No. of patients with adequate treatment	21	8	21	22	72
No. of complete responses	4(19%)	0	0	1(4%)	5
No. of partial responses	11(52%)	4(50%)	10(48%)	8(36%)	33
No. of minor responses	5(24%)	0	5(24%)	7(32%)	17
No. of no responses	1(5%)	4(50%)	6(29%)	7(32%)	18
No. of complete responses + partial responses	15(71%)	4(50%)	10(48%)	9(40%)	38
Total No. of patients evaluable for toxicity*	33	11	21	48	113
Maximum creatinine level (mg/100 ml)					
≤2	28	8	19	47	102
>2	5†	3‡	2§	1¶	11
Wbc nadir (cells × 10^3/mm^3)					
3.0–3.9	7	6	4	8	25
2.0–2.9	4	3	4	8	19
<2.0	1	1	6	0	8
Platelet nadir (cells × 10^3/mm^3)					
100–150	9	2	3	4	18
50–99	0	2	9	4	15
<50	1	5	3	3	12
Stomatitis	9	8	5	0	22
Hearing loss	3	1	0	0	4

*Total No. of patients evaluable for toxicity includes previously treated patients.
†Maximum creatinine levels of > 10, 6, 4, 3.8, and 2.8 mg/100 ml.
‡Maximum creatinine levels of 16, 4.8, and 2.4 mg/100 ml.
§Maximum creatinine levels of 2.4 and 2.2 mg/100 ml.
¶Maximum creatinine level of 2.1 mg/100 ml.

Regimen C

After the disappointing results with regimen B, we decided to lower the dose of methotrexate and to add vinblastine, an infrequently used agent in head and neck cancer but one with demonstrated activity (8). We also shortened the bleomycin infusion from 7 to 4 days and placed the *cis*-platinum at the end of the infusion, to be able to give the methotrexate before the *cis*-platinum and to decrease the total time required for chemotherapy administration from 10 days (regimen A) and 14 days (regimen B) to 5 days. We had hoped that whatever toxicity would result from this four-drug regimen would be largely dissipated by 2–3 weeks; the patient could then receive either surgery or radiation therapy.

As shown in table 1, although 15 of the 21 patients were stage IV on presentation, surgery played a central role in the subsequent treatment of 13 of the 21. Since our criteria of operability have not changed during the course of these trials, it is clear that as the trials have progressed the patient characteristics have shifted significantly in favor of those patients with less bulky disease. As can be seen in table 2, the activity of this combination did not appear greater than the activity of regimen A. Although the nephrotoxicity of this combination, with its slightly lower *cis*-platinum dose and its markedly lower methotrexate dose, was quite acceptable, the myelosuppression was rather severe, with ten of 21 patients showing wbc nadirs of $< 3000/mm^3$ and 12 of 21 patients showing platelet nadirs of $< 100{,}000/mm^3$. Those patients with wbc counts of $< 2000/mm^3$ uniformly developed fever during the neutropenic period and were given iv antibiotics. Although there were no deaths due to therapy among this group of patients, the myelosuppression, often occurring with stomatitis, was responsible for much morbidity and prolongation of hospitalization time. Only an occasional patient was ready to receive radiation therapy or a surgical procedure before Day 28. At present, the followup time is too short to allow analysis of the long-term results.

Regimen D

Since the addition of more drugs to regimen A had only served to increase toxicity without any suggestion of an increase in therapeutic effect, it seemed most pertinent to examine the effect of *cis*-platinum alone in previously untreated patients. As can be seen in table 2, the re-

sponse rate of 40% seems suggestively lower than the response rate of 71% obtained with regimen A in, if anything, a more advanced group of patients. Responses were near maximal after only one dose of *cis*-platinum; one patient who achieved a minor response after one dose became a partial responder after the second, and a second patient who was a partial responder after the first dose continued to improve and achieved complete remission after a total of three doses. No patient received more than three doses. It should be noted that our practice of giving further drug to those patients showing some response to regimen D would tend to maximize the response rate of this treatment in comparison to the others, where evaluation was made at a fixed point in the trial. Thus, the apparent superiority of regimen A becomes more impressive. Interestingly, the observed activity of *cis*-platinum in previously untreated patients is not significantly different from the response rate (30%) in the very heavily pretreated patients that we studied in the initial phase II trial (5).

The toxicity of *cis*-platinum alone in the previously untreated group was mild: one patient had a creatinine level of > 2 mg/100 ml and none of the patients had platelet counts of $< 50{,}000/mm^3$ or wbc counts of $< 2000/mm^3$. To indicate our overall experience with the toxicity of *cis*-platinum alone in head and neck cancer patients, the data from the current group of 22 previously untreated patients have been combined with those of the 26 patients with heavy prior therapy previously reported (5), and the results are shown in table 2.

DISCUSSION

A few conclusions are possible from the data presented above. *cis*-Platinum, alone or in combination, produces major degrees of tumor regression in 40%–70% of previously untreated patients with head and neck cancer. The addition of bleomycin by continuous infusion to *cis*-platinum results in a higher regression rate than we have achieved with *cis*-platinum alone. The further addition of high-dose methotrexate or conventional doses of methotrexate and vinblastine to *cis*-platinum and bleomycin, in the doses and schedules shown in figure 1, is associated with unacceptable degrees of toxicity and no discernible increase in activity, although the small number of patients treated with regimen B precludes any accurate assessment of response rate.

Although *cis*-platinum is clearly useful in head and neck cancer, its administration by the technique used here (4) is not convenient, involving hospitalization for at least 48 hours/treatment. In addition, it produces nausea and vomiting of such a degree as to constitute a potential stumbling block for patients requiring repeated courses of treatment. The Southwest Oncology Group has recently been exploring an outpatient *cis*-platinum regimen consisting of a dose of 50 mg/m^2 iv on Days 1 and 8, with recycling once a month. Although the study is not completed, the preliminary data show no obvious evidence of cumulative nephrotoxicity at early followup times;[3] the activity of this dose and schedule of *cis*-platinum appears roughly equivalent to that reported in our phase II evaluation (5). Corder et al (9) have described a regimen of *cis*-platinum including an osmotic diuresis but designed for outpatient use. This program appears practical for those with day-hospital facilities but has not yet been tested for activity in head and neck cancer. In an attempt to reduce the complexity of this regimen, we employed the same dose and schedule of *cis*-platinum (40 mg/m^2/week) but without prehydration or a forced diuresis (10). Although four of 13 patients with head and neck cancer had either a complete or partial response, dose-limiting nephrotoxicity occurred in 50% of patients.

Jacobs et al (11) have studied 24-hour infusions of *cis*-platinum accompanied by vigorous hydration but no mannitol. Among 18 patients (six previously untreated) usually treated with 80 mg/m^2/24 hours, the response rates (complete response + partial response = 39%; complete response + partial response + minor response = 72%) are identical to those we found with *cis*-platinum given by bolus at a somewhat higher dose. With the 24-hour infusion, nephrotoxicity was minimal and gastrointestinal upset was absent in eight of 32 courses.

Hong et al (12) have used *cis*-platinum and bleomycin as described above in a similar series of patients. Their data on activity and toxicity are virtually superimposable on ours. Jacobs[4] has attempted to combine infusions of *cis*-platinum with methotrexate at doses of 200–1000 mg/m^2 followed by leucovorin. More myelosuppression occurred with this combination than with *cis*-platinum alone, and there was no obvious evidence of therapeutic synergism, although this was a small series. Elias et al (13) have utilized high-dose *cis*-platinum, bleomycin by continuous infusion, and high-dose methotrexate in combination as a preoperative adjuvant. No increase in response rate over that reported for *cis*-platinum and bleomycin seems to have occurred; the toxicity of their three-drug combination was not notably different from that of *cis*-platinum and bleomycin.

Since the use of chemotherapy as initial treatment in a multimodality effort is still rather new, all involved groups have been concerned with the possibility of an increased incidence of complications following any subsequent surgery or radiation therapy. Although such data are still rather a mass of impressions, there have been no intraoperative or postoperative problems attributable to the chemotherapy. Wounds appear to hold together much as they did without chemotherapy pretreatment, and postoperative infections seem to occur with about the

[3]Panettiere FJ. Personal communication.

[4]Jacobs C. Personal communication.

same frequency as always. The surgical specimen, following an impressive response to chemotherapy, may be largely necrotic, but viable-appearing tumor cells are usually very much in evidence. Shapshay et al (14) have noted some increase in the amount of keratinization after chemotherapy, compared with the original histologic appearance of the biopsy specimen prior to drug administration. The acute toxicity of a course of therapeutic irradiation following chemotherapy appears to be indistinguishable from that seen with radiation alone. Whether the potential long-term side effects of radiation will also be unchanged is another matter; needless to say, there are now no available data.

It is, of course, true that initial chemotherapy need not be *cis*-platinum-based chemotherapy. Tarpley et al (15) have had much experience with the use of methotrexate in this situation; further recent work with this drug used singly at high doses (16) shows activity at least equivalent to that of *cis*-platinum. Price et al have explored the effects of a complex combination of agents in both previously treated (17) and untreated (18) patients with head and neck cancer. The regimen is clearly an active one, although when allowance is made for the rather large number of unevaluable patients, it is difficult to say whether the regimen appears more active than either *cis*-platinum or methotrexate. The apparent absence of toxicity with the regimen of Price et al is noteworthy and further followup information on the disease-free survival of this patient group is awaited with interest.

The critical question, of course, is whether the use of chemotherapy as initial treatment has any effect on the natural history of this group of diseases. Specifically, what is the effect of these treatments on total and disease-free survivals? Is the pattern of relapse affected? Is the frequency of appearance of second primary cancers altered? What is the chronic toxicity of these treatments? Such questions are not easily approached by uncontrolled studies involving limited numbers of patients. For this reason, the National Cancer Institute (Bethesda, Md) is supporting a cooperative study involving several institutions and cooperative groups. The study will compare standard therapy (surgery and radiation) with two experimental programs in a randomized fashion: (*a*) standard therapy preceded by chemotherapy with *cis*-platinum and bleomycin; and (*b*) standard therapy preceded by chemotherapy with *cis*-platinum and bleomycin and followed by maintenance chemotherapy for 6 months with *cis*-platinum by 24-hour infusion once a month. It is only by such studies, involving a simultaneous control group and large numbers of patients, that the issues raised by pilot studies can be settled definitively.

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cis-Dichlorodiammineplatinum(II) for the Treatment of Advanced Ovarian Cancer

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SUMMARY

***cis*-Dichlorodiammineplatinum(II) (*cis*-platinum) was used in the treatment of 25 patients with advanced ovarian adenocarcinoma refractory to alkylating agents. Seven of 24 evaluable patients (29%) responded to *cis*-platinum (one complete response and six partial responses). Life-table analysis of survival indicates that patients responding to therapy survive longer than those who fail to respond (9 months versus 3.2 months) ($P = 0.014$). In previously treated patients given 70 mg/m^2 iv with forced diuresis, there was substantial nausea and vomiting (100%), hematologic toxicity (67%), and renal toxicity (34%). A review of the single-agent activity of *cis*-platinum in other ovarian cancer studies indicates an overall response rate of 25% (40 of 161 patients). At least nine combination chemotherapy studies utilizing *cis*-platinum are in progress, and preliminary information from several of these is encouraging and suggests that *cis*-platinum-containing combinations may have a major role in the treatment of ovarian adenocarcinoma.**

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Ovarian carcinoma is the fifth most frequent cause of death from cancer among women in the United States and the most common cause of death from a gynecologic cancer (1). The first signs and symptoms of the disease are vague, which often precludes early diagnosis, and approximately two thirds of patients initially present with advanced disease outside of the true pelvis (stages III and IV according to the International Federation of Gynecology and Obstetrics). In addition, 20%–40% of women with what appears to be localized disease will develop recurrent disease which will require further therapy (2,3). Therefore, most patients with ovarian carcinoma eventually need chemotherapy for their disease.

The drugs currently considered active (response rates of $\geq 20\%$) in ovarian cancer are as follows: melphalan, thioTEPA, cyclophosphamide, chlorambucil, mechlorethamine, 5-fluorouracil, methotrexate, hexamethylmelamine, Adriamycin, and *cis*-dichlorodiammineplatinum(II) (*cis*-platinum). Despite the use of some of these agents alone or, more recently, in combination regimens (4), the overall complete remission rates in ovarian cancer and the survival of patients with advanced disease have been less than desired. In particular, when a patient fails to respond to the original chemotherapeutic regimen, it is difficult to induce second remissions. The report from The Royal Marsden Hospital (Sutton, Surrey, England) establishing the activity of *cis*-platinum in previously treated ovarian cancer patients has been an important addition to the treatment of ovarian cancer (5,6). This report was of particular interest in light of the pharmacologic information about *cis*-platinum gained from animal studies (7). *cis*-Platinum is retained at high

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concentrations in the ovaries of beagle dogs for as long as 6 days after drug administration.

Our purpose in this report is to detail the clinical trials with *cis*-platinum alone in ovarian cancer, both at the Medicine Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, and at other institutions. In addition, we will discuss the current protocols which include *cis*-platinum in combination with other drugs considered active in ovarian carcinoma. Hopefully, this information will provide areas worthy of further clinical development.

In this review a complete response (CR) is defined as the disappearance of all clinical disease for at least 4 weeks. A partial response (PR) is defined as a decrease in the size of palpable tumor by >50% for at least 4 weeks from the start of therapy or a reduction of ascites and/or pleural effusions requiring no further paracentesis for at least 3 months. In most of the studies reviewed, the assessment of the extent of response has been clinical only. Restaging with laparoscopy or "second-look" laparotomy has been employed in very few studies. This results in variable CR, PR, and overall response rates.

SINGLE-AGENT ACTIVITY

The Medicine Branch began clinical trials with *cis*-platinum in 1975. Twenty-five patients with ovarian cancer were entered in a phase II trial (table 1). All patients had refractory stage III or IV ovarian cancer. All had been heavily pretreated with an average of four drugs, and one had received prior radiotherapy to major marrow-bearing sites. Median age and other characteristics of the population are detailed in table 1.

Following 12 hours of iv hydration and 60 minutes after 40 mg of furosemide and 37.5 g of mannitol administration, *cis*-platinum was infused iv over 30–60 minutes at a dose of 70 mg/m^2. Following *cis*-platinum administration, patients received additional infusions of 5% dextrose in ½ N saline plus 50 g of mannitol and 40 mg of KCl/liter run at 200 ml/hour × 5 hours followed by 12 hours of iv hydration. The regimen was repeated every 21 days. Creatinine clearances, complete chemistry panels, cbcs, and audiograms were performed prior to each chemotherapy course. Blood and urine levels of *cis*-platinum were also determined in 11 patients.

The 25 patients entered in the study received a total of 110 courses of *cis*-platinum. Twenty-four patients were evaluable for response and toxicity. The one nonevaluable patient had no measurable disease. One patient achieved a CR and was still in CR at the 31-day followup examination. Six patients achieved a PR with *cis*-platinum, with a median duration of response of 4 months (range, 3–8.5 months). Thirteen patients had stable disease and four of the 24 patients had progressive disease. The median number of days it took to achieve a response was 42 (range, 21–135). Figure 1 shows the survival curves for all evaluable patients entered in the study categorized by type of response (CR + PR versus no response + progressive disease). A one-tailed test was used to determine whether those who achieved a CR or PR lived longer, and the difference was significant at $P = 0.014$ (Gehan's test). The median survival time was 9 months for those who responded to *cis*-platinum, while those who did not respond had a median survival of 3 months. These results are gratifying since all patients studied had been heavily pretreated. Previous studies suggest that significant responses after the failure of initial therapy are rare in this disease (8).

TABLE 1.—Phase II study of *cis*-platinum in advanced ovarian carcinoma: patient characteristics

Characteristics	
Total No. of patients	25
No. of evaluable patients	24
No. of patients with tumor stage	
III	19
IV	5
Age at entry in study	
Median	52 yrs
Range	28–64 yrs
No. of patients with prior therapy	
Chemotherapy alone	7
Surgery + chemotherapy	16
Surgery + chemotherapy + radiotherapy	1
Total No. of courses completed	110
Median per patient (range)	3 (1–21)
Median time from first histologic diagnosis to entry in study (range)	16 mos (7–90)
Median duration of CR (1 patient)	1+ mo
Median duration of PR (6 patients) (range)	3.7 mos (3–8.5)
Median No. of days from first dose to date of PR (range)	42 (21–135)

The toxicity of the treatment included nausea and vomiting which were severe in 80% of the patients; however, no patient discontinued treatment because of nausea and vomiting. In 17% of the patients renal toxicity was shown by serum creatinine levels of 2.1–3.5 mg/100 ml and an additional 17% had serum creatinine levels of > 3.5 mg/100 ml. All of these changes were reversible. Thirteen percent of the courses were associated with wbc counts of < 1000/mm^3 and in an additional 67% of the courses patients had wbc counts of < 3000/mm^3. Thrombocytopenia was also very prevalent in these heavily pretreated patients. Four percent of the courses produced platelet counts of < 25,000/mm^3 and an additional 45% of the courses produced platelet counts of < 50,000/mm^3. Elevations in liver function tests were occasionally noted but

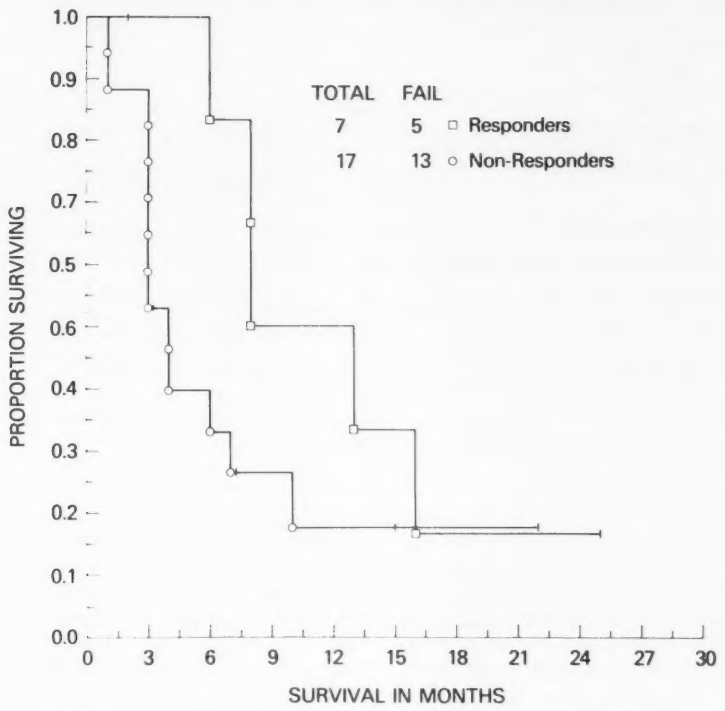

FIGURE 1.—*cis*-Platinum treatment of advanced ovarian cancer: survival of responders and nonresponders.

were difficult to separate from elevations caused by the patient's disease. Reversible clinical ototoxicity was noted in only one patient. Peripheral neuropathy secondary to *cis*-platinum was seen in a single patient (9).

The regimen was tolerable but toxic. Maximum attention to nephrotoxicity from *cis*-platinum was essential. Bone marrow toxicity was significant and was probably, in part, secondary to the heavy pretreatment that these patients received.

Of additional interest are the pharmacologic studies performed on 11 patients receiving *cis*-platinum. As noted in table 2, there was no substantial difference in pharmacologic parameters for responders and nonresponders to *cis*-platinum therapy. Our study in this limited number of patients does not correlate well with the preclinical studies, which have suggested that tumor response is a direct function of duration of exposure to *cis*-platinum (10).

The experience of other institutions and cooperative groups with *cis*-platinum as a single agent in ovarian cancer is detailed in table 3. There have been ten other phase II studies reported. Most of the patients treated with *cis*-platinum in these studies had been refractory to alkylating agents and, with the exception of patients in one study, all had received prior chemotherapy or prior chemotherapy plus radiotherapy. Treatment schedules and doses were variable. Most regimens, whether high- or low-dose, included some type of forced diuresis. Daily × 5 days or continuous infusion schedules have not been reported for ovarian cancer. Response rates in these studies have ranged from 5% to 35%, with an overall response rate in 161 patients of 25%. Median survivals, where specified, have ranged from 1 to 22 months. It is of note that of the 40 recorded responses, only two have been CRs.

TABLE 2.—Pharmacokinetic measurements for *cis*-platinum

	Responders (3 patients)	Group average* (8 patients)
Peak plasma level (μM)	31.4 ± 8.6	30.4 ± 5.9
8-hr plasma level (μM)	15.0 ± 4.3	12.9 ± 1.6
12-hr plasma level (μM)	14.1 ± 3.9	14.0 ± 2.8
4-hr urine excretion (%)	20.3 ± 0.8	17.7 ± 2.5
Fast-phase half-life (mins)	66 ± 18	65 ± 22
Slow-phase half-life (hrs)	57.7 ± 13	56 ± 10.6

*3 responders and 5 nonresponders.

TABLE 3.—Studies utilizing *cis*-platinum alone for ovarian carcinoma*

Institution (ref No.)	Dose (mg/m^2)	Schedule	No. of remissions/ No. of evaluable patients	% response rate
The Royal Marsden Hospital (6)	30	qd × 3, q4wks	3/13	27
	30	q2–3wks	5/19	
Southwest Oncology Group (11)	75	q3wks	2/15	13
National Cancer Institute (present study)	70	q3wks	7/24	29
Mt. Sinai Hospital† (12)	50	q3wks	6/17	35
Mt. Sinai Hospital (13)	50	q3wks	6/19	32
Roswell Park Memorial Institute (14)	50–100	q3wks	1/20	5
Gynecologic Oncology Group (15)	50	q3wks	10/34	29

*Data are confined to phase II studies with > 10 patients.
†No prior therapy.

The conclusion from the single-agent data is that *cis*-platinum is an active agent in ovarian cancer patients who have failed to respond to prior chemotherapy. The collected data do not show any trend suggesting an advantage of one regimen over another. The usefulness of the drug in the face of clinical resistance to alkylating agents contradicts the suggestions from the preclinical data that animal tumors resistant to cyclophosphamide were also resistant to *cis*-platinum (16).

CIS-PLATINUM IN COMBINATION REGIMENS FOR OVARIAN CARCINOMA

With the known activity of *cis*-platinum as a single agent in human ovarian carcinoma, a number of combination regimens have been developed (table 4). These regimens have some preclinical rationale because *cis*-platinum is synergistic with a wide variety of compounds, including cyclophosphamide in animal tumor systems (24–26). Some of the regimens listed in table 4 are used for newly diagnosed ovarian cancer and some are used for refractory cancer. Currently, none of the treatment programs listed in table 4 have been proven more effective than *cis*-platinum alone either in previously treated or previously untreated patients. The early response rates in these studies, however, are promising. At this time, results are far too preliminary to recommend one regimen over another.

The Medicine Branch is conducting an ongoing study in previously untreated advanced ovarian cancer patients with a drug combination called CHex-UP. This combination includes cyclophosphamide (150 mg/m^2 orally on Days 2–7 and 9–16), hexamethylmelamine (150 mg/m^2 orally on Days 2–7 and 9–16), 5-fluorouracil (600 mg/m^2 iv on Days 1 and 8), and *cis*-platinum (30 mg/m^2 on Days 1 and 8) with prior hydration and mannitol-induced diuresis. To date, 19 patients with stage III and IV ovarian cancer have been accrued. Twelve of these patients are evaluable for response. Three of the 12 patients are in CR, documented by restaging laparoscopy and laparotomy, and seven are at least in PR, with five of the seven still having decreasing disease. Two patients have not achieved a response. While these results are preliminary, and the number of patients is small, they are encouraging and will be followed closely. It should be emphasized that this regimen is toxic and dose modifications are required depending upon individual tolerance.

DISCUSSION

From the information presented it is clear that *cis*-platinum is an active single agent for the treatment of refractory ovarian cancer. Patients responding to *cis*-platinum in the National Cancer Institute study have survived longer than nonresponders. The drug's activity in previously untreated patients has not been extensively studied; however, it is active in refractory cases and is now being employed in several combination regimens. The optimal dose schedule for *cis*-platinum administration is unknown at this time. High-dose ($\geq$ 100 mg/m^2) regimens have not been compared in a randomized fashion to low-dose (< 100 mg/m^2) regimens. It is also not known whether a patient who fails to respond at a lower dose will or will not respond to a higher dose of the drug.

In our experience and in the experience of other investigators, the drug is reasonably easy to handle provided attention is paid to the nephrotoxicity (pretreatment hydration is a necessity) and provided hematologic support is available. Hematologic toxicity has been much greater in our experience than previously reported. This

TABLE 4.—Combination regimens including *cis*-platinum for the treatment of ovarian carcinoma

Institution (ref No.)	Drugs used	No. of evaluable patients	% overall response rate
Mt. Sinai Hospital (12)	*cis*-Platinum + Adriamycin	18	66
Cancer and Acute Leukemia Group B* (17)	*cis*-Platinum + Adriamycin	24	38
The Royal Marsden Hospital (18)	*cis*-Platinum + chlorambucil	35	60
Albert Einstein College of Medicine† (19)	*cis*-Platinum + hexamethylmelamine	5	80
Albert Einstein College of Medicine† (19)	*cis*-Platinum + hexamethylmelamine + Adriamycin	12	50
Central Pennsylvania Oncology Group (20)	*cis*-Platinum + hexamethylmelamine + Adriamycin + cyclophosphamide	22	50
Albert Einstein College of Medicine (21)	*cis*-Platinum + hexamethylmelamine + Adriamycin + cyclophosphamide	12	50
Mt. Sinai Hospital† (22)	*cis*-Platinum + Adriamycin + cyclophosphamide	24	50
University of Indiana (23)	*cis*-Platinum + Adriamycin + cyclophosphamide	29	72
National Cancer Institute (present study)	*cis*-Platinum + hexamethylmelamine + cyclophosphamide + 5-fluorouracil	12	83

*Some patients had prior treatment and some did not.
†All patients had prior treatment.

is presumably due to the heavy prior therapy our patients have received. However, the initial experience with *cis*-platinum in combination therapy suggests that significant increases in bone marrow toxicity may be expected when the drug is included in a combination.

Early results of combination regimens including *cis*-platinum for the treatment of ovarian cancer are particularly promising and await further clinical trials. Other areas for possible further work include testing several of the *cis*-platinum analogs against ovarian cancer (27), utilizing the drug ip by dialysis (28), as has been done for methotrexate and 5-fluorouracil, and, possibly, because of preclinical evidence that *cis*-platinum can act as a radiosensitizer (29,30), exploring the use of *cis*-platinum plus radiotherapy in resistant cases of ovarian cancer. It is readily apparent that *cis*-platinum will be a major part of future treatment regimens for advanced ovarian cancer.

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Cancer of the Ovary: A Summary of Experience With *cis*-Dichlorodiammineplatinum(II) at The Royal Marsden Hospital [1]

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SUMMARY

A review of the use of *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) as a single agent in 82 patients with advanced ovarian carcinoma, previously treated with chemotherapy, shows that response rates of 33% and 52% are achieved with doses of 30 and 100 mg/m^2 respectively. In 58 previously untreated patients a combination of chlorambucil and *cis*-platinum (regimen B) was compared in a randomized study with a combination of chlorambucil, *cis*-platinum, and Adriamycin (regimen C). Complete responses were seen in 32% and 41% of the patients respectively. Remissions were most prolonged in patients with complete regressions, the median being > 15 months for both regimens. Because of the good regressions, second-look operations have been possible in 12 patients for the purpose of confirming regression and performing radical surgical removal. In six of these patients, all specimens failed to show evidence of residual carcinoma. The major toxic effects of *cis*-platinum in our hands are neurologic effects and anemia; both have been reversible after cessation of treatment.

[Cancer Treat Rep 63:1545–1548, 1979]

In 1971 we first became aware that a new compound, *cis*-dichlorodiammineplatinum(II) (*cis*-platinum), might be of value in the treatment of malignant disease. The late Prof. Sir Alexander Haddow encouraged us to test the compound because, although it appeared to have an action similar to that of an alkylating agent, it was the first potential antitumor drug containing a metal. Clinical studies were started in 1972 and by good fortune we chose to use *cis*-platinum in carcinoma of the ovary. The choice was a fortunate one, since by 1974 only two tumors were known to respond to the compound; these were testicular and ovarian tumors.

At that time we reported responses in patients with extensive disease who had relapsed, or had not responded, to other chemotherapy and we were impressed by the responses we saw. We were pleased that we had not seen renal damage, which was frequently noted by other workers. The lack of renal toxicity may well have been due to the fact that, from the beginning of our investigations, we gave 12-hour iv hydration after each injection of *cis*-platinum, although the drug was often given in very low doses. These results encouraged us to continue our clinical studies at a time when others felt that *cis*-platinum was too dangerous to be of great value. We report here a review of our work in 140 fully evaluable patients with ovarian adenocarcinoma.

Advanced, Previously Treated Disease

Our initial studies were done on patients with advanced disease, many of whom had had radiotherapy to the pelvis and all of whom were resistant to other chemotherapy. A total of 52 patients were treated and 34 of them have been previously reported (1). Twenty-three patients received 30 mg/m^2 daily on 3 successive days every 4 weeks, and the rest received 30 mg/m^2 on 1 day every 3 weeks. The response rates were 26.5% and 28% respectively. The overall response rate was 33% with a complete response rate of 6%. Responses were maintained for a median of 4 months. We considered that, in this small series, there was no therapeutic advantage to the larger dose.

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[1] *cis*-Dichlorodiammineplatinum(II) was supplied by the National Cancer Institute, Bethesda, Md.

[2] The Royal Marsden Hospital, London, England.

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Following the work of Hayes et al (2), we began a separate study in a similar group of patients using the high-dose regimen of 100 mg/m^2 once monthly, with pretherapy and posttherapy hydration and mannitol diuresis. Of 30 fully evaluable patients the overall response rate was 52% with 16% complete responses. Seven of these patients had had previous exposure to low-dose *cis*-platinum and of these, two patients responded (one complete and one partial). Both remissions lasted 5 months. Of those patients with no previous exposure to *cis*-platinum, complete responses have continued for as long as 27 months, with a median of > 12 months. Partial remissions were shorter, with a median of 6 months. The median survival was 6 months for nonresponders, 8 months for partial responders, and > 12 months for complete responders. Because of toxicity, to be described later, after five courses of high-dose *cis*-platinum the drug has been discontinued and remissions are maintained using hexamethylmelamine in intermittent courses.

Advanced, Previously Untreated Disease

Because of the encouraging results with low-dose *cis*-platinum in preterminal cases, a randomized trial was started comparing chlorambucil alone given in intermittent courses of 0.2 mg/kg/day for 14 days every 28 days (regimen A) with chlorambucil given at a dose of 0.15 mg/kg/day for 7 days together with *cis*-platinum (20 mg/m^2) on Day 1 of the course (regimen B). This treatment was repeated at 3-week intervals. The patients in the study had not received previous chemotherapy and either had FIGO stage III or IV disease at the time of operation or had recurrent disease following pelvic or pelvic and abdominal radiotherapy. The study is a collaborative one, with patients being entered by two other hospitals, but only our own patients are reported here.

Early in this trial the results with chlorambucil alone were so disappointing that regimen A was abandoned. At that time, 16 patients had received regimen A and there were only four partial regressions. The trial was continued, comparing regimen B with chlorambucil plus *cis*-platinum, given as before, plus Adriamycin (50 mg/m^2) on Day 1 (regimen C).

At present, there are 58 evaluable patients in the study: 34 in regimen B and 24 in regimen C. Combination chemotherapy is continued for 12 months, although Adriamycin is discontinued when a total of 500 mg/m^2 has been administered. Maintenance chemotherapy is then given for a further 12 months. This consists of chlorambucil alone, 0.2 mg/kg/day for 14 days every month.

When treatment was started, all patients had measurable disease and no "debulking" operative procedures had been done. The overall response rates were 59% for regimen B and 66% for regimen C. Complete regressions occurred in 32% and 41% respectively (table 1). These responses were measured by clinical examination, radiology, and ultrasound techniques. More recently, second-look operations have been done for two reasons: first, to perform a radical operation which has not been done at the diagnostic laparotomy and to remove any residual macroscopic disease elsewhere, and second, to better assess the degree of response. The indication for this operation is a complete clinical or a good partial regression of tumor after five courses of combination chemotherapy. The laparotomy is performed between courses 6 and 7, and chemotherapy is continued even if histologically all disease has apparently been eliminated. So far, 12 operations have been performed. Four patients received regimen B and eight received regimen C. Seven patients were in complete clinical remission at the time and six of them had no histologic evidence of residual tumor despite the fact that the omentum, the residual ovarian tissue, and the uterus were removed and multiple biopsies of the peritoneum and liver were performed. Gross tumor was seen at operation in three patients who have since relapsed 6, 8, and 12 months after operation (table 1). The remaining patients continue in remission for 4–21 months after the procedure.

Remissions from the start of chemotherapy for both regimens B and C are short if only partial regressions are obtained. The median duration is 5 months for regimen B and between 6 and 9 months for regimen C. If remissions are complete, the median durations are > 15 months with either regimen. The longest remission so far is 30 months and this is continuing.

In regimen B the survival of complete responders is > 18 months (the longest so far is 34 months), while the nonresponders have a median survival of 13 months. It should be noted that when the *cis*-platinum combination fails, the patients then go on to receive the HexaCAF regimen[4] of Fisher and Young (3), and although, at that stage of the disease, measurable responses are rare, the treatment may be prolonging survival.

Possible Factors Influencing Response Rates

Factors which might influence response to *cis*-platinum include the patient's age, the histologic degree of differentiation, the stage of the disease, and previous treatment.

We looked at the response rates among patients treated with regimens B and C and found that there was an overall response rate of 43% for those ≤ 50 years of age, 66.5% for those between 51 and 60 years of age, and 72% for those > 60 years of age. However, the total number of patients in each group is small (14, 24, and 20 respectively).

Stage of disease is clearly of importance when considering the value of a chemotherapy schedule; it is clear

[4]HexaCAF = hexamethylmelamine, cyclophosphamide, Adriamycin, and 5-fluorouracil.

TABLE 1.—Characteristics of patients receiving chlorambucil plus *cis*-platinum with (regimen C) or without (regimen B) Adriamycin

	Regimen B	Regimen C
No. of patients treated	34	24
Stage at start of chemotherapy (No. of patients)		
III	17	13
IV	6	7
Recurrent disease	11	4
No. of responses		
Complete	11 (32%)	10 (41%)
Partial	9 (27%)	6 (25%)
Median length of complete response	15 mos	15 mos
No. of 2nd-look operations	4	8
No. of patients with no pathologic evidence of tumor	3	3
No. of patients who relapsed following operation	2	1

that at low doses of *cis*-platinum (20–30 mg/m^2) the response rates are low in patients previously treated with other chemotherapy. However, high-dose *cis*-platinum (100 mg/m^2) has a response rate of 52% which compares very favorably with that of reported combination regimens in earlier stage disease (table 2). Nevertheless, complete remissions were seen less frequently.

Regimens B and C were given to patients immediately after operation (stages III and IV) or after recurrence following radiotherapy for stages I and II disease. Responses were seen in 53% of 15 patients who were treated for recurrence, 66% of 30 patients with stage III disease, and 61% of 13 patients with stage IV disease. The numbers are, as yet, too small to know whether previous radiotherapy really reduces the response rates.

Review of histology is not yet complete in our study, but so far there is little difference in the responses of well-differentiated, moderately differentiated, and poorly differentiated tumors. If anything, more responses are seen in the moderately differentiated group.

Side Effects and Toxicity

Hematologic toxicity was only a problem when radiotherapy and chemotherapy had been given previously, and it is not our practice to scale down doses when blood cell counts are low. We prefer to postpone therapy until the counts have recovered and then give full doses. Postponement very rarely occurred except in those patients receiving the high-dose regimen. However, one hematologic effect which occurred in about 25% of the patients was persistent normochromic normocytic anemia which

TABLE 2.—*cis*-Platinum versus combination chemotherapy in patients previously treated with other chemotherapy

Authors (ref No.)	Regimen	No. of patients treated	% response		Overall response rate (%)
			Complete	Partial	
Wiltshaw et al (partially reported in ref 1)	*cis*-Platinum, 30 mg/m^2	52	6	27	33
Wiltshaw et al (this report)	*cis*-Platinum, 100 mg/m^2	30	16	36	52
Bruckner et al (4)	Cyclophosphamide, Adriamycin, and *cis*-platinum	24	8	42	50
Vogl et al (ECOG)* (6)	Hexamethylmelamine, Adriamycin, and *cis*-platinum	27	18	48	66
Kane et al (5)	Cyclophosphamide, hexamethylmelamine, Adriamycin, and *cis*-platinum	39	18	26	44

*ECOG = Eastern Cooperative Oncology Group.

continued throughout the *cis*-platinum treatment period; about half of these patients required blood transfusions. Anemia was reversed after *cis*-platinum was stopped.

Nephrotoxicity was seen in only three patients, two receiving high-dose therapy and one receiving regimen C. We were surprised by this lack of toxicity and believe that the incidence may depend on the general condition of the patient before the drug is given, the amount of disease affecting the renal tract itself, and the degree of hydration before and after therapy.

With the high-dose regimen we began to observe a series of neurotoxic side effects which we had not seen with the low-dose regimen. Eight of 24 patients who received three or more doses of 100 mg/m^2 developed neurologic complications of varying degree. These included severe peripheral neuropathy, unexplained seizures, blurred vision, and hearing loss. The drug was stopped immediately when neurotoxicity was suspected and it is now our practice to give no more than five consecutive doses of the high-dose regimen and to follow this with hexamethylmelamine as a single agent until relapse occurs. Fortunately, all the symptoms and signs of neurologic damage have disappeared with time, although in one case this took as long as 6 months.

DISCUSSION

In comparing the results of chemotherapy in ovarian cancer it is now essential to know if extensive cytoreductive surgery has been carried out before the drugs are given and what criteria are being used to assess complete remission. None of our patients had had aggressive surgery before *cis*-platinum therapy and all had clinically measurable disease. These patients had a large tumor burden and the response rates are relatively high, particularly with the high-dose regimen, when compared with other agents. Our responses are measured by clinical, radiologic, and ultrasound monitoring, and have been confirmed by laparotomy or laparoscopy only in a minority of patients.

Recently, further reports have appeared on the usefulness of *cis*-platinum in ovarian cancer. In patients resistant to previous chemotherapy, Bruckner et al (4) reported a response rate of 50% using a cyclophosphamide, Adriamycin, and *cis*-platinum (50 mg/m^2) regimen. Under similar circumstances, Kane et al (5) reported seven complete remissions and ten partial remissions among 39 patients receiving a combination of cyclophosphamide, hexamethylmelamine, Adriamycin, and *cis*-platinum, while Vogl et al reported an Eastern Cooperative Oncology Group study (6) in which five complete and 13 partial remissions were seen among 27 patients. In their study, hexamethylmelamine, Adriamycin, and *cis*-platinum were used in combination. Interestingly, these combination chemotherapy regimens give response rates remarkably similar to ours using high-dose single-agent *cis*-platinum therapy (table 2).

Unfortunately, there has been no study of single-agent *cis*-platinum in previously untreated patients with ovarian tumors. Such a study should be done, since it is clear that combination chemotherapy will prove more expensive and will probably result in more side effects and toxicity. At the present time, our study comparing chlorambucil and *cis*-platinum with or without additional Adriamycin does not show a statistically significant difference in response rates or survival; however, more time is needed before final conclusions can be made.

CONCLUSIONS

cis-Platinum is one of the most useful drugs in the treatment of advanced ovarian cancer and should now be incorporated into the general management of this disease. This drug, in combination with aggressive surgical removal of all visible tumor, may well lead to improvement in the cure rate of this highly malignant and relatively radioresistant tumor.

The limiting toxic effect, in our hands, has been neurologic rather than renal, but high-dose therapy may be very dangerous in the long term if given repeatedly.

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cis-Dichlorodiammineplatinum(II) in the Treatment of Gynecologic Malignancies: Phase II Trials by the Gynecologic Oncology Group [1]

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SUMMARY

A dose of 50 mg/m^2 of *cis*-dichlorodiammineplatinum(II) was administered iv every 3 weeks to 25 patients with squamous cell carcinoma of the cervix and to 34 patients with adenocarcinoma of the ovary. All patients had advanced or recurrent disease. Eleven of the 25 patients (44%) with cervical carcinoma had an objective response while ten of 34 patients (29%) with ovarian carcinoma demonstrated an objective response. Toxic effects were frequent but tolerable, with no drug-related deaths and only minimal drug-related nephrotoxicity, except in one patient. Myelosuppression was more frequent in the more heavily previously treated ovarian carcinoma patients. *cis*-Dichlorodiammineplatinum(II) thus appears to be a highly active single agent in the treatment of squamous cell carcinoma of the cervix and adenocarcinoma of the ovary.

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Among the common gynecologic malignancies, significant data identifying highly active chemotherapeutic agents are available only for ovarian adenocarcinoma (1). In recognition of this deficiency, the Gynecologic Oncology Group (GOG) initiated a broad phase II study of newer agents with an emphasis on evaluation against squamous cell carcinoma of the uterine cervix, adenocarcinoma of the ovary, and adenocarcinoma of the endometrium. *cis*-Dichlorodiammineplatinum(II) (*cis*-platinum) was selected for this trial. This report presents results of the use of *cis*-platinum in the treatment of squamous cell carcinoma of the cervix and adenocarcinoma of the ovary.

MATERIALS AND METHODS

Twenty-six patients with histologically confirmed squamous cell carcinoma of the cervix and 37 patients with histologically confirmed adenocarcinoma of the ovary from nine GOG member institutions were entered in this study to receive *cis*-platinum. All patients had advanced or recurrent disease which was no longer amenable to control with surgery, radiation therapy, or more standard chemotherapy. Patients were treated with 50 mg/m^2 of *cis*-platinum iv at a rate of 1 mg/minute every 3 weeks. Patients were hospitalized for 12 hours before therapy and 24 hours after therapy to receive iv fluids at a rate of 100 ml/hour. No mannitol was given. Therapy was continued until disease progression or until toxic effects occurred requiring discontinuation of therapy.

Among the 26 patients with squamous cell carcinoma, 25 were considered to be evaluable for response and tox-

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icity. One patient had evidence of marked renal insufficiency at the time of entry and was thus ineligible. The 25 evaluable patients all had measurable disease, normal renal function (as measured by a BUN of < 25 mg/100 ml and a creatinine of < 1.5 mg/100 ml), and adequate blood cell counts (as determined by a granulocyte count of > 2000/μl and a platelet count of > 100,000/μl). Hepatic function, as documented by a normal SGOT, alkaline phosphatase, and total bilirubin, was also normal. All patients had received prior therapy, although only four of the 25 had received prior chemotherapy, with none having received prior *cis*-platinum. Twenty-four patients had received prior radiotherapy, 16 had received prior surgery, and one had received prior hormonal therapy. This patient population represents a fair trial of the efficacy of *cis*-platinum as evidenced by the low frequency of prior chemotherapy and the fact that 20 patients had a performance status (PS) of 0 or 1 while the remaining patients had a PS of 2 according to the following GOG PS scale:

0 = fully active.
1 = strenuous activity restricted, ambulatory.
2 = unable to work, capable of self-care, ambulatory, up 50% of waking hours.
3 = limited self-care, confined to bed or chair > 50% of waking hours.
4 = completely disabled, no self-care.

Among the 37 patients with adenocarcinoma of the ovary, 34 were considered to be evaluable for response and toxicity. Two patients never received the drug and it was considered too early to evaluate the course of one patient. As with the 25 evaluable patients with squamous cell carcinoma of the cervix, these 34 evaluable patients all had measurable disease, normal renal and hepatic function, and adequate blood cell counts. All of the patients had also received prior therapy. Thirty-three patients had had prior surgery, 13 had had prior radiotherapy, and two had had prior hormonal therapy. In contrast to the patients with cervical carcinoma, however, 30 of the 34 patients with ovarian adenocarcinoma had received prior chemotherapy. In spite of the frequency of prior chemotherapy, this patient population probably represents a fair trial of the efficacy of *cis*-platinum in ovarian adenocarcinoma since 26 patients had PS 0 or 1 and only eight had PS 2 or 3.

The criteria used to gauge response and toxicity are reasonably standard among the cooperative oncology groups. Response was gauged by objective measurement of documented disease. A *complete response* (CR) was defined as a disappearance of all gross evidence of disease for at least 1 month, a *partial response* (PR) was defined as a ≥ 50% reduction in the products obtained from measurement of perpendicular diameters of each lesion with a duration of reduction of at least 1 month, and *progressive disease* was defined as a ≥ 50% increase in the product obtained from measurement of perpendicular diameters of any lesion or the appearance of any new lesion. *Stable disease* was defined as disease not meeting any of the above criteria. *Survival* was defined as the observed length of life from entry in the study until death or, for living patients, date of last contact. *Progression-free interval* was defined as the time from entry in the study to the date of reappearance of or documentation of increasing disease parameters or to the date of last contact. This category was used for patients with stable or progressive disease. *Response duration* was defined as the time from documentation of objective response to the date of reappearance of or documentation of increasing disease parameters or to the date of last contact.

Hematologic and renal toxic effects were graded as indicated in table 1. Other toxic effects are listed as mild, moderate, or severe or are described fully.

RESULTS

Antitumor Activity

Cervical Carcinoma

The response of the 25 evaluable patients with squamous cell carcinoma of the cervix is shown in table 2.

TABLE 1.—Grading of hematologic and renal toxicity

	Grade				
	0	1	2	3	4
Wbcs/μl	≥ 4000	3000–3999	2000–2999	1000–1999	< 1000
Granulocytes/μl	≥ 1500	1000–1499	500–999	250–499	< 250
Platelets/μl	≥ 150,000	100,000–149,999	50,000–99,999	25,000–49,999	< 25,000
BUN (mg/100 ml)	≤ 20	21–40	41–60	> 60	
Creatinine (mg/100 ml)	≤ 1.2	1.3–2.0	2.1–4.0	> 4.0	
Creatinine clearance (ml/min)	> 50	35–50	20–34	< 20	

TABLE 2.—Response to *cis*-platinum in 25 patients with squamous cell carcinoma of the cervix

	Response				
	CR	PR	Stable disease	Progression	Totals
No. of patients (%)	3 (12)	8 (32)	12 (48)	2 (8)	25 (100)
No. of courses to response					
Median	3	2.5	—	—	3
Range	2–3	1–6	—	—	1–6
No. of courses received					
Median	6	6	3.5	3.5	4
Range	4–8	3–9	2–8	2–5	2–9
Time to response (mos)					
Median	2	2	—	—	2
Range	1–3	1–4.5	—	—	1–4.5
Response duration (mos)					
Median	5	4+	—	—	4.5
Range	4+–6	2–7	—	—	2–7
Survival (mos)					
Median	7+	7+	3.5	6+	6.5+
Range	6+–8+	4.5–9+	2–6	4–8+	2–9+

TABLE 3.—Responses of pelvic versus extrapelvic disease in patients with squamous cell carcinoma of the cervix

Metastatic disease site	No. of patients				
	CR	PR	Stable disease	Progression	Totals
Pelvis only	2	4	8	1	15
Outside pelvis	1	4	4	1	10
Totals	3	8	12	2	25

Only 8% of patients demonstrated continued disease progression in the first month of therapy, whereas 44% responded with a ≥ 50% reduction in tumor volume. Response, when observed, was prompt, with a median time from initiation of therapy to response of 2 months (range, 1–4.5 months) or three drug courses (range, one to six courses) (table 2). Thus, the greater number of courses received by responders was most likely a result of rather than a cause of response. The median duration of responses was 4.5 months with responders surviving significantly longer than nonresponders ($P = 0.01$) (table 2). Frequency of response could not be related to factors including age, time from disease onset to recurrence, histologic grade, PS, site of metastatic disease, and prior therapy. With particular reference to site of metastatic disease, responses were evenly distributed between patients with pelvic disease only and those with disease in distant sites (table 3).

Ovarian Adenocarcinoma

The response of the 34 evaluable patients with adenocarcinoma of the ovary is presented in table 4. Only 15% of patients demonstrated continued disease progression in the first month of therapy, whereas 29% responded with a ≥ 50% reduction in tumor volume. As with cervical carcinoma, response was prompt and occurred after a median of 2 months (range, 1–4 months) or two drug courses (range, one to five courses) (table 4). Information regarding response duration and survival of responders is incomplete as yet. Response rate could not be related to factors including age, time from disease onset to recurrence, histologic grade, site of metastatic disease, and prior therapy. Although not statistically significant, response by PS is interesting in that there were ten responses in 26 patients with PS 0 or 1 and no responses in eight patients with PS 2 or 3 (table 5). It is also interesting that all of the responses were seen in patients

TABLE 4.—Response to *cis*-platinum in 34 patients with adenocarcinoma of the ovary

	Response				
	CR	PR	Stable disease	Progression	Totals
No. of patients (%)	2 (5.9)	8 (23.5)	19 (55.9)	5 (14.7)	34 (100)
No. of courses to response					
Median	2.0	2.5	—	—	2.0
Range	1–5	2–3	—	—	1–5
No. of courses received					
Median	6.0	5.5	4.0	2.0	4.0
Range	1–6	2–9	2–11	1–3	1–11
Time to response (mos)					
Median	2	2	—	—	2
Range	2, 2	1–4	—	—	1–4
Response duration (mos)					
Median	2.5+	2+	—	—	2+
Range	2+–3+	1–4.5+	—	—	1–4.5+
Survival (mos)					
Median	3.5+	5.5+	4.5	3	4.5
Range	3+–4+	4+–7+	1+–8+	1–5	1–8+

TABLE 5.—Response according to PS in patients with ovarian carcinoma

	Response				
PS	CR	PR	Stable disease	Progression	Totals
0	1	1	6	1	9
1	1	7	7	2	17
2	0	0	5	2	7
3	0	0	1	0	1
4	0	0	0	0	0
Totals	2	8	19	5	34

who had received prior chemotherapy; none of the four patients who had not received prior chemotherapy responded.

Toxicity

Toxic effects were frequent but tolerable in patients with carcinoma of the cervix. Myelosuppression was manifested by leukopenia in seven patients and thrombocytopenia in nine patients (table 6). In the seven patients with leukopenia, the median wbc count nadir was 3200/μl (range, 1900–3900/μl) and the median number of drug courses until the nadir occurred was three (range, one to seven courses). In the nine patients with thrombocytopenia, the median platelet count nadir was 116,000/μl (range, 15,000–143,000/μl) and the median number of drug courses until the nadir occurred was two (range, one to nine courses). These adverse hematologic effects occurred in a total of 11 of the 25 patients, with only one of the 11 experiencing life-threatening toxicity (platelet count of 15,000/μl). Nephrotoxicity was surprisingly mild, with only four of 25 patients experiencing more than minimal elevations of BUN and creatinine (table 7). Three of these four patients had progressive pelvic disease with evidence of ureteral obstruction as an explanation for increasing azotemia. The remaining patient developed acute renal failure 1 week after the first dose of *cis*-platinum which necessitated hemodialysis; no subsequent recovery of renal function occurred. The only other frequent toxic effect was gastrointestinal (table 7), with 17 patients experiencing mild to moderate nausea and vomiting, six experiencing severe problems, and two having no gastrointestinal difficulty. Less frequent problems included fever (two patients with temperature of 100°F–101°F), local phlebitis at the injection site (one patient), severe hypocalcemia (one patient with calcium < 6 mg/100 ml), and life-threatening anaphylaxis (one patient). There were no drug-related deaths.

TABLE 6.—Myelosuppression in 25 patients with squamous cell carcinoma of the cervix treated with *cis*-platinum

Toxicity grade	No. of patients				
	CR	PR	Stable disease	Progression	Totals
			*Leukopenia**		
0	1	4	11	2	18
1	2	3	0	0	5
2	0	1	0	0	1
3	0	0	1	0	1
4	0	0	0	0	0
Totals	3	8	12	2	25
			*Thrombocytopenia**		
0	2	4	9	1	16
1	1	2	1	1	5
2	0	2	1	0	3
3	0	0	0	0	0
4	0	0	1	0	1
Totals	3	8	12	2	25

*Median wbc count nadir: 3200/μl (range, 1900–3900/μl). Median No. of courses to wbc count nadir: 3 (range, 1–7). Median platelet count nadir: 116,000/μl (range, 15,000–143,000/μl). Median No. of courses to platelet count nadir: 2 (range, 1–9). 11 of the 25 patients had leukopenia and/or thrombocytopenia.

TABLE 7.—Gastrointestinal and renal toxicity of *cis*-platinum in patients with squamous cell carcinoma of the cervix and adenocarcinoma of the ovary

Toxicity grade	No. of patients with toxicity	
	Gastrointestinal	Renal
	*Squamous cell carcinoma of cervix**	
0	2	9
1	1	12
2	16	2
3	6	0
4	0	2
Totals	25	25
	Adenocarcinoma of ovary†	
0	0	17
1	1	17
2	25	0
3	8	0
4	0	0
Totals	34	34

*Gastrointestinal toxicity was manifested by nausea and vomiting which was moderate in most patients. Nephrotoxicity was mild or absent in all except 4 patients, 3 of whom had disease-related rather than drug-related renal impairment.

†Gastrointestinal toxicity was manifested by nausea and vomiting which was moderate in most patients, while drug-related nephrotoxicity was minimal.

Toxic effects were also frequent but tolerable in the 34 patients with adenocarcinoma of the ovary. Myelosuppression was manifested by leukopenia in 24 patients and thrombocytopenia in 18 patients (table 8). In the patients with leukopenia, the median wbc count nadir was 3000/μl (range, 200–3700/μl) with only two patients demonstrating a wbc count of < 2000/μl. In the patients with thrombocytopenia, the median platelet count nadir was 125,000/μl (range, 8000–149,000/μl) with only one patient demonstrating a platelet count of < 40,000/μl. Nephrotoxicity was again surprisingly minimal, with one half of the patients experiencing no renal impairment and one half experiencing minimal rises in BUN and creatinine (table 7). The only other frequent toxic effect was gastrointestinal, with 26 patients experiencing mild to moderate toxic effects and eight experiencing severe nausea (table 7). Other toxic effects noted include mild alopecia (one patient), mild myalgias (one patient), confusion (one patient), hypokalemia and trismus which was severe (one patient), and anemia requiring transfusion (one patient). There were no drug-related deaths.

DISCUSSION

cis-Platinum is the first of a number of heavy metal complexes to reach clinical trial as an antineoplastic agent in human malignancy. The present study does not represent the first clinical trial of the drug as a single agent in the treatment of gynecologic malignancies. In a broad phase II trial of *cis*-platinum in a dose schedule of 15–25 mg/m^2/day × 5 days every 3–4 weeks, five PRs among 20 patients with advanced ovarian carcinoma were noted (2). These results were subsequently confirmed in another report of nine responses among 34 patients with advanced ovarian carcinoma treated with either a high-dose schedule of 30 mg/m^2/day × 3 days every 3 weeks or a low-dose schedule of 30 mg/m^2 as a single dose every

3 weeks (3). No advantage was noted for the high-dose schedule over the low-dose regimen. Subsequently, the Southwest Oncology Group reported two responses in 15 patients with advanced carcinoma of the ovary as well as no responses in four patients with carcinoma of the cervix to a single dose of *cis*-platinum of 75 mg/m^2 every 3 weeks (4). These data suggest that *cis*-platinum may be an active agent in the treatment of ovarian carcinomas. Too little data are available to indicate efficacy in squamous cell carcinoma of the cervix and adenocarcinoma of the endometrium.

The current GOG study was undertaken to confirm the activity of *cis*-platinum in the treatment of ovarian carcinoma and to evaluate the activity against squamous cell carcinoma of the cervix and adenocarcinoma of the endometrium. Not enough cases of endometrial adenocarcinoma have been accrued as yet to evaluate drug activity against this neoplasm. Results noted, however, confirm the activity of *cis*-platinum as a second-line drug in the treatment of ovarian carcinoma. The response rate of 29% in this study is virtually identical to that reported in two other studies (2,3). More significant are the data indicating a high level of activity for *cis*-platinum as a first-line agent in the treatment of squamous cell carcinoma of the cervix. The response rate of 44% far exceeds that reported for any other single agent (1) and is similar to that reported for successful combination chemotherapy (5). The duration of response, furthermore, exceeds that reported for the combination of mitomycin C, vincristine, and bleomycin.

Toxic effects were frequent in both patient populations. The greater frequency of myelosuppression in the patients with ovarian carcinoma almost certainly relates to the fact that most of these patients, in contrast to those with cervical carcinoma, had received prior chemotherapy (most with melphalan) prior to entry in this study. The relative infrequency of serious drug-related nephrotoxicity probably reflects the low dose of *cis*-platinum employed in this trial. This low dose probably also accounts for the lack of any neurotoxicity noted in the study population. As of this report, no patient has received more than nine courses (450 mg/m^2) of drug and hence the likelihood of serious neurotoxicity would be expected to be reasonably low.

In view of the significant response rates in ovarian and cervical carcinoma with the relatively low dose of *cis*-platinum employed, serious questions are raised regarding the use of high-dose schedules which are more toxic and require intensive diuresis to prevent significant nephrotoxicity (6). The general use of such high-dose schedules should come only after documentation of enhanced efficacy, documentation which is as yet lacking (3).

In conclusion, *cis*-platinum is clearly a highly active agent in the treatment of squamous cell carcinoma of the cervix and adenocarcinoma of the ovary. Studies of *cis*-platinum in combination with Adriamycin and alkylating agents suggest an even higher order of activity for such combinations in the treatment of carcinoma of the ovary (7,8). Future efforts, thus, would appear to involve confirming the high level of activity for *cis*-platinum against squamous cell carcinoma of the cervix and further ex-

TABLE 8.—Myelosuppression in 34 patients with adenocarcinoma of the ovary treated with *cis*-platinum

Toxicity grade	No. of patients				
	CR	PR	Stable disease	Progression	Totals
			Leukopenia *		
0	1	1	6	2	10
1	1	4	5	3	13
2	0	2	7	0	9
3	0	1	0	0	1
4	0	0	1	0	1
Totals	2	8	19	5	34
			Thrombocytopenia †		
0	0	5	8	3	16
1	2	2	7	2	13
2	0	1	2	0	3
3	0	0	1	0	1
4	0	0	1	0	1
Totals	2	8	19	5	34

*24 of the 34 patients had leukopenia with only 2 patients having a wbc count < 2000/μl. Median wbc count nadir: 3000/μl (range, 200–3700/μl).

†18 of the 34 patients had thrombocytopenia with only 1 patient having a platelet count of < 40,000/μl. Median platelet count nadir: 125,000/μl (range, 8000–149,000/μl).

ploiting *cis*-platinum combinations in the treatment of carcinoma of the ovary. Initial data on *cis*-platinum as therapy for endometrial carcinoma will also be forthcoming.

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Phase II Evaluation of *cis*-Dichlorodiammineplatinum(II) in Advanced Malignancies of the Genitourinary and Gynecologic Organs: A Southwest Oncology Group Study [1]

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SUMMARY

One hundred and forty-seven fully and partially evaluable patients with advanced measurable malignancies of the genitourinary and gynecologic organs were given *cis*-dichlorodiammineplatinum(II) at a dose of 75 mg/m^2 iv every 3 weeks. Thirty-six patients with testicular neoplasms were studied; five complete responses (13.9%) and seven partial responses (PR) (19.4%) were noted. Thirty-seven patients with ovarian adenocarcinoma were evaluated; five PRs (13.5%) were seen. One complete response (11.1%) and two PRs (22.2%) were obtained among nine patients with urinary bladder cancer. Four PRs (19.0%) were seen among a group of 21 patients with advanced prostate cancer. One PR (4.8%) was noted among 21 patients with renal cell cancer and no responses were seen in eight patients with cervical cancer. There was a highly statistically significant ($P < 0.001$) survival advantage for the responding testicular tumor patients. Toxicity was similar to that previously reported, with gastrointestinal side effects and nephrotoxicity most commonly seen. Prospective and sequential analysis of renal function provided strong evidence for cumulative nephrotoxicity in these patients given bolus injections of *cis*-dichlorodiammineplatinum(II) without prehydration or treatment with furosemide or mannitol.

[Cancer Treat Rep 63:1557–1564, 1979]

In an effort to determine the activity of *cis*-dichlorodiammineplatinum(II) (*cis*-platin) in advanced malignancies of the genitourinary and gynecologic organs, in 1974 the Southwest Oncology Group (SWOG) initiated a phase II study of this compound given at a dose of 75 mg/m^2 iv every 3 weeks. It is the purpose of this report to present the results of this study in terms of response rates, remission durations, survival, and toxicity.

MATERIALS AND METHODS

Eligibility criteria included at least one measurable lesion in a patient with an advanced or metastatic surgically incurable, histologically verified malignant neoplasm of a genitourinary or gynecologic organ, an anticipated survival of $\geq$ 8 weeks, complete recovery from toxicity due to previous chemotherapy and/or radiotherapy, a patent urinary tract confirmed by an iv pyelogram, an absolute granulocyte count of $\geq$ 2000/μl, a platelet count of $\geq$ 100,000/μl, a BUN of $\leq$ 20 mg/dl, and an endogenous creatinine clearance of $\geq$ 75 ml/minute. All patients had either disease recurrence after prior treatment or diseases for which there are no accepted standard treatments. Patients with prostate cancer were also eligible

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TABLE 1.—Response rates to *cis*-platin given at a dose of 75 mg/m² iv every 3 wks

Tumor site	No. of patients*	No. of responses (%)		
		CR	PR	Improvement
Testicle	36	5 (13.9)	7 (19.4)	3 (8.3)
Ovary	37	0	5 (13.5)	2 (5.4)
Bladder	9	1 (11.1)	2 (22.2)	
Prostate	21	0	4 (19.0)	
Kidney	21	0	1 (4.8)	
Cervix	8†	0	0	

*All fully and partially evaluable patients with a malignancy of the given organ site.
†7 patients with epidermoid carcinoma and 1 with adenocarcinoma.

TABLE 2.—Medians and ranges of remission durations (wks) for responding patients in this study, analyzed according to type of response and site of primary tumor

Tumor site	Response category	Remission duration (wks)	
		Median	Range
Testicle	CR	45	5–83+
	PR	15	6–124+
	CR + PR	15	5–124+
Ovary	PR	7	3–20
Bladder	CR + PR	3	2–10
Prostate	PR	14	4–15

TABLE 3.—Median survivals (wks) following *cis*-platin treatment

Tumor site and response category	Median survival (wks)	Survival rates (%)		*P* value
		6 mos	12 mos	
Testicle				
CR + PR	70	92	58	<0.001
Other	21	27	0	
Ovary				
PR	21	38	0	0.31
Other	17	34	14	
Bladder				
CR + PR	30	67	33	0.43
Other	12	24	0	
Prostate				
PR	90+	75	75	0.21
Other	21	45	0	
Kidney				
All patients	27	52	7	—
Cervix				
All patients	24	44	44	—

if they had established osseous metastases and elevated serum acid phosphatase levels. Written consent forms were provided by all patients, consistent with the policies of the institutions where they were treated and evaluated.

cis-Platin was diluted to a concentration of 1 mg/ml and was given as a rapid iv infusion. No effort was made to prehydrate the patients and forced diuresis with mannitol or furosemide was not attempted.

Patients with stable disease or improvement continued to receive the drug at 3-week intervals. An adequate trial of treatment is considered to be two injections of *cis*-platin with the associated 6-week observation period. Each week, patients had cbcs, platelet counts, and measurements of BUN, serum creatinine, and serum uric acid. Before each course of therapy and at the conclusion of the study, a 24-hour endogenous creatinine clearance and audiometry were assessed.

Complete response (CR) is defined as the complete disappearance of all measurable disease. Partial response (PR) is defined as a reduction by 50% in the product of the largest diameter of each measurable lesion and its perpendicular. Improvement is defined as tumor regression of insufficient magnitude to qualify as PR. For patients with prostate cancer, a PR is defined as a $\geq 50\%$ decrease in the serum acid phosphatase level with no progression of symptoms or appearance of new lesions.

One hundred and fifty-seven of 160 patients entered in this study were eligible. One hundred and twenty-two patients are fully evaluable. Twenty-five patients are only partially evaluable: 13 because of early death, four because they had an inadequate trial due to toxicity, three because they refused further therapy, two because they were lost to followup, and three for various other reasons. Ten patients are not evaluable: six because of a major protocol violation, three because insufficient data were provided, and one for another reason.

RESULTS

Tumor Responses

Response rates are summarized in table 1. Among testicular tumor patients, responses were seen in pure seminomas, embryonal carcinomas, teratocarcinomas, choriocarcinomas, and tumors of mixed histology. Other responses included a PR in one of three patients with fallopian tube carcinoma and a PR in the single patient with a retroperitoneal (extragonadal) embryonal carcinoma. Other treatment failures included two patients with endometrial carcinoma, two with squamous cell carcinoma of the vagina, three with carcinomas of the renal pelvis, and one each with carcinomas of the ureter, urethra, and adrenal cortex. Two other treatment failures, not included in this final analysis, were noted in two patients with gynecologic sarcomas.

Remission durations (in weeks), recorded as medians and associated ranges, are shown in table 2.

Survival

Table 3 summarizes certain features of the survival data. In general, the numbers of patients involved are small, so that few significant comparisons would be expected.

The survival curve for testicular tumor patients with PR is similar to that for patients with CR ($P = 0.66$). These curves are shown in figure 1. When the data on these 12 patients are pooled and compared with the data on testicular tumor patients with all other responses (improvement, stable disease, or progressive disease), there is a highly statistically significant difference between the curves ($P < 0.001$) (fig 2).

Toxicity

The degree and frequencies of gastrointestinal, renal, and hematopoietic toxic effects are shown in table 4. In addition, two episodes each of convulsions, tinnitus, and hearing loss were reported.

Our data were analyzed further in order to determine if *cis*-platin caused cumulative renal damage. Figure 3 shows the mean creatinine clearances by course and by number of courses administered to a total of 76 patients from this study. There clearly seems to be a trend toward declining renal function as additional courses of *cis*-platin are administered.

Eleven patients had creatinine clearances determined both prior to course 1 and prior to course 6. The mean creatinine clearances prior to courses 1 and 6 were 123 and 60 ml/minute respectively. Using the paired t test, this drop of 63 ml/minute is highly significant at the $P = 0.01$ level.

Figure 4 shows the mean creatinine clearances ($\pm$ 2 SEM) plotted against the given course number for all patients. Again, a trend toward declining renal function is evident.

DISCUSSION

Our data support the high order of activity for *cis*-platin in testicular tumors noted by Higby et al (1), Hayes et al (2), and Merrin (3). Our results are pooled with those of Higby et al in table 5. The overall CR, PR, and improvement rates are 23.5%, 19.6%, and 13.7% respectively. The overall CR plus PR rate is 43.1%, with 95% confidence limits of 29.4%–57.8%. If improvement patients are included, the overall response rate is 70.6%, with 95% confidence limits of 43.3%–70.7%. Hayes et al (2) reported "useful antitumor effects" in six of nine patients with testicular tumors. Merrin (3) noted a CR in five of nine patients treated with *cis*-platin and an

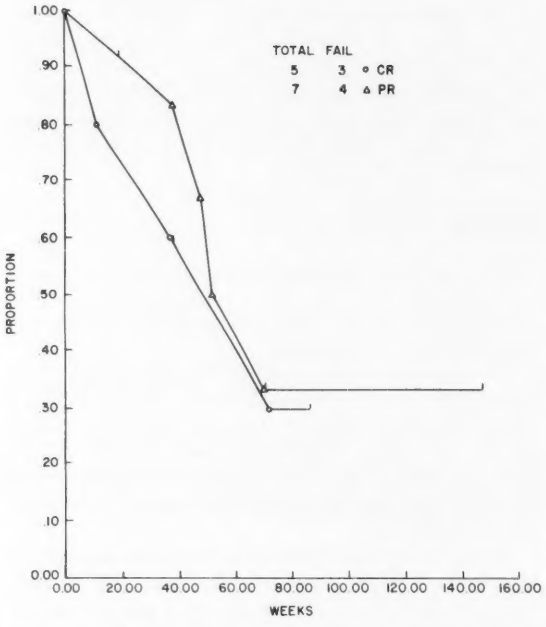

FIGURE 1.—Survival curves comparing patients with advanced testicular tumors treated with *cis*-platin: CR vs PR. (*See* text for details.)

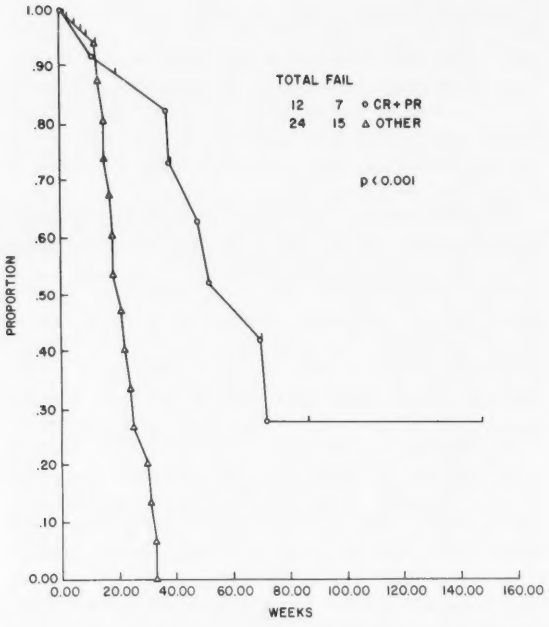

FIGURE 2.—Survival curves comparing patients with advanced testicular tumors treated with *cis*-platin: CR + PR vs all other responses. (*See* text for details.)

TABLE 4.—Toxicity analysis in 147 evaluable patients categorized according to Southwest Oncology Group criteria

Type of toxicity	Grade 1 (mild)	2 (moderate)	3 (severe)	4 (life-threatening)	5 (fatal)	Totals
Gastrointestinal, nausea/vomiting	11*	38*	33*			82*
Renal						
BUN (mg/dl)	21–40	41–60	61–100	>100		
Creatinine (mg/dl)	1.2–2.0	2.1–4.0	4.1–6.0	>6.0		
Creatinine clearance (ml/min) (% of pretreatment value)	>75%	50%–74%	25%–49%	<25%		
	26*	10*	11*	2*		49*
Hematopoietic						
Wbcs/μl or	3000–3999	2000–2999	1000–1999	<1000		
Absolute polymorphonuclear cells/μl	1001–1500	501–1000	251–500	≤250		
	21*	16*	3*	1*		41*
Platelets/μl	75,000–99,999	50,000–74,999	15,000–49,999	<25,000		
	9*	4*	6*	3*		22*

*No. of patients.

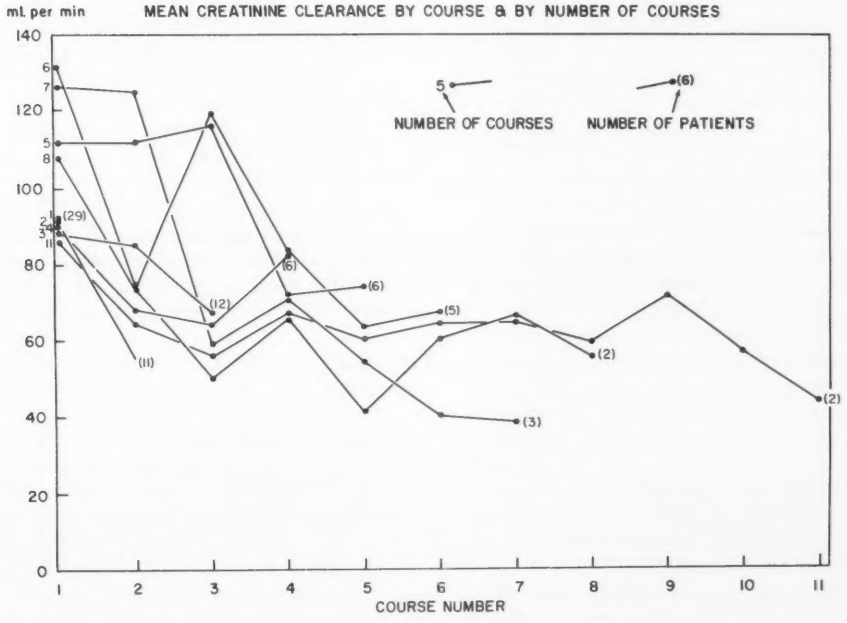

FIGURE 3.—Mean creatinine clearances by course and by No. of courses for 76 patients for whom these data are available. (*See* text for details.)

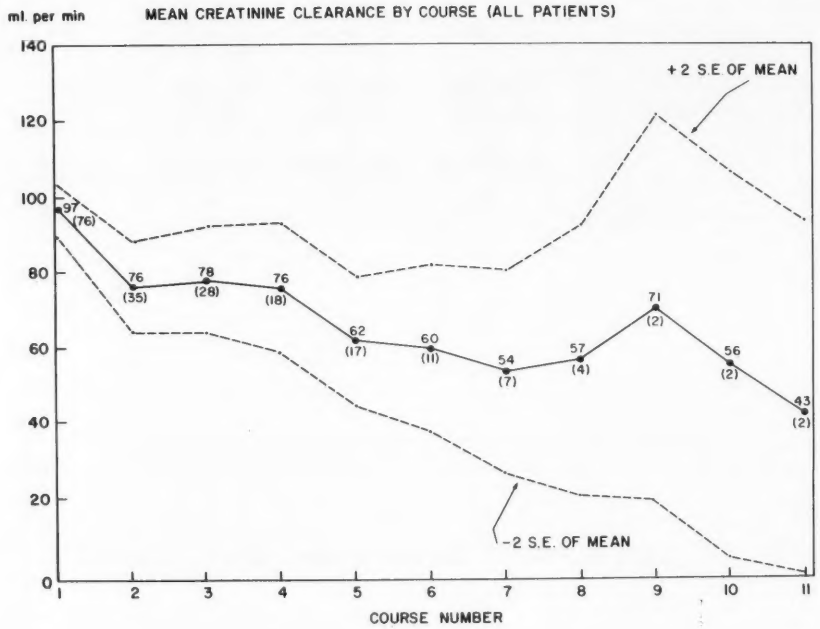

FIGURE 4.—Mean creatinine clearance by course, ± 2 SEM. The No. above each data point represents the value of the calculated mean creatinine clearance, while the No. below the data point in parentheses indicates the No. of patients studied at that course number and contributing to the mean creatinine clearance calculation.

TABLE 5.—Cumulative data on reported responses of metastatic testicular tumors to *cis*-platin (the 95% confidence limits of the calculated response rates [CR + PR] for 51 patients are 29%–58%)

Reference	No. of patients	No. of responses		
		CR	PR	Improvement
Southwest Oncology Group (this study)	36	5	7	3
Higby et al (1)	15	7	3	4
Totals	51	12	10	7

TABLE 6.—Cumulative data on reported responses of advanced ovarian adenocarcinoma to *cis*-platin (the 95% confidence limits of the calculated response rates [CR + PR] for 110 patients are 12%–28%)

Reference	No. of patients	No. of responses		
		CR	PR	Improvement
Southwest Oncology Group (this study)	37	0	5	2
Wiltshaw and Kroner (4)	34	1	8	—
Bruckner et al (5)	19	0	6	—
Piver et al (6)	20	1	0	—
Totals	110	2	19	2

"objective response" in an additional three patients. Our survival data comparing CR plus PR patients in this series (table 3 and fig 2) indicate a highly significant ($P < 0.001$) advantage for responding patients.

Table 6 contains pooled data from this study and from three other studies which evaluated the activity of *cis*-platin as a single agent in advanced adenocarcinoma of the ovary (4–6). CRs were rare, occurring in only two of the 110 patients, but 19 PRs (17.3%) have been noted. The survival data from our study for the patients with ovarian carcinomas are not impressive (table 3). In another study, however, the median survival time was 12 months for responding patients compared to 6 months for nonresponding patients (4).

Two studies in addition to ours have evaluated the activity of *cis*-platin in advanced carcinoma of the urinary bladder (7,8). Among the total of 52 patients, there were two CRs, 18 PRs, and four improvements, confirming the activity of this compound against this tumor. These data are summarized in table 7. Our survival data for these patients do not indicate an important survival advantage for responders (table 3).

Merrin (9) has observed PRs in nine of 21 patients (42.8%) with advanced prostate cancer treated with *cis*-platin. Some of his reported responses are based on rather "soft" data such as mobilization of a pleural effusion or resolution of extremity edema. In addition, the bulk of his measurable lesions were in soft tissues, not the typical and common pattern of metastases for prostate cancer. We have observed four PRs (19.0%) among 21 patients with advanced prostatic cancer, all occurring in patients with advanced osteoblastic metastases and elevated serum acid phosphatase levels. Although not highly significant statistically, there is a consistent trend favoring the survival characteristics of the responding patients compared to the nonresponders.

We observed one PR among 21 patients with advanced renal cell carcinoma treated with *cis*-platin alone. This low order of response is consistent with the observation by Rodriguez and Johnson (10) who noted only one improvement among 23 evaluable patients.

The toxic effects noted by us are quite consistent with those reported previously in phase I trials (11,12). Among the patients with renal cell carcinomas, no apparent differences were noted in the toxicity characteristics when comparing those patients who had a nephrectomy with those who did not.

From the current study, we have been able to gather strong evidence indicating cumulative nephrotoxicity with repeated courses of *cis*-platin. These observations are best shown in figure 3, which is similar to figure 3 in the paper by Dentino et al (13). It should be recalled that neither we nor Dentino et al made a specific effort to provide prehydration or diuresis to our patients prior to receiving the *cis*-platin. Prehydration and diuretic therapy may circumvent this problem (2,3,14), but the

TABLE 7.—Cumulative data on reported responses of advanced urinary bladder cancer to *cis*-platin (the 95% confidence limits of the calculated response rates [CR + PR] for 52 patients are 25%–53%)

Reference	No. of patients	No. of responses		
		CR	PR	Improvement
Southwest Oncology Group (this study)	9	1	2	
Yagoda et al (7)	24	0	8	4
Merrin et al (8)	19	1	8	—
Totals	52	2	18	4

effect of this procedure still requires further documentation.

We conclude that this new inorganic antineoplastic agent has important activity against human malignancies of the testicle, ovary, bladder, and prostate. The gastrointestinal and renal toxic effects associated with this compound, when used at the dosage and schedule employed in this study, suggest that the ultimate role of this agent might be at a reduced dosage in combinations using other active drugs. The potential hazard of cumulative nephrotoxicity must be considered when designing studies integrating this compound into adjuvant settings. The search for safer methods of administration and for active, less toxic congeners must continue.

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Phase II Trials With *cis*-Dichlorodiammineplatinum(II) in the Treatment of Urothelial Cancer [1]

Alan Yagoda [2,3,*]

SUMMARY

Results of disease-oriented phase II trials with *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) in 135 adequately treated patients with advanced urothelial tumors at Memorial Sloan-Kettering Cancer Center are presented. In four protocols which used *cis*-platinum alone or in combination with Adriamycin and/or cyclophosphamide in 95 patients with bladder cancer, no significant difference (46%–54%) in the number of partial remissions (PRs) in previously untreated patients was noted. The median duration of response in three of the four protocols was 5–7 months. A review of the literature indicates that *cis*-platinum used singly produced remissions in 45% of 67 patients (95% confidence limit, 12%–57%). In the treatment of superficial bladder tumors, intravesically administered *cis*-platinum induced few complete or sustained remissions. The difficulties in evaluating response with intravesical therapy are discussed. The importance of patient selection, particularly the need to include patients with objectively measurable disease parameters, in phase II trials is stressed. Differences in patient characteristics and response criteria will necessitate prospective randomized trials of *cis*-platinum alone versus *cis*-platinum combination regimens in the treatment of metastatic disease. *cis*-Platinum was inactive (12% PRs) in 25 patients with prostatic cancer who had objectively measurable parameters. It is of interest that PRs were obtained in three of six patients (50%) with penile cancer. A review of the literature and the data in the present series indicates that *cis*-platinum has no value in the treatment of metastatic hypernephroma.

[Cancer Treat Rep 63:1565–1572, 1979]

cis-Dichlorodiammineplatinum(II) (*cis*-platinum) has produced significant tumor regression in germ cell tumors of the testis in early phase I–II trials (1). In one study, Wallace and Higby (1) noted a complete remission (CR) of > 1 year's duration in a patient with bladder cancer treated with *cis*-platinum and suggested that *cis*-platinum may be an effective drug in tumors which originate in the genitourinary ridge. Notably, Fisher et al (2) have described a response to *cis*-platinum in a patient with metastatic cloacogenic carcinoma of the anus.

This paper updates the results of phase II disease-oriented trials (3–5)[4] (tables 1 and 2) at Memorial Sloan-Kettering Cancer Center (MSKCC) with *cis*-platinum used singly and in combination regimens in the treatment of some tumors of the genitourinary anlage. Disease sites include the kidney, renal pelvis, ureter, urinary bladder, urethra, prostate, and penis.

MATERIALS AND METHODS

Each patient had a complete medical history and physical examination, and accessible lesions were measured

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TABLE 1.—Disease-oriented phase II *cis*-platinum protocols at MSKCC

Tumor	Protocol	Drug(s)	Dose* (mg/m^2)	Day
Bladder				
Metastatic	I	*cis*-Platinum	50–70	1
	II	*cis*-Platinum	70	1
		CYC	250–1000	1
	III	*cis*-Platinum	70	1
		Adriamycin	30–60	1
	IV	*cis*-Platinum	70	1
		CYC	250	2
		Adriamycin	45	3
Local	A	*cis*-Platinum	60–70	1
	B	*cis*-Platinum	1 mg/ml (intravesical)	
Prostate		*cis*-Platinum	45–70	1
Kidney		*cis*-Platinum	120 or 50–70	1
Penis		*cis*-Platinum	120	1

* All iv doses were given q3–4wks. Intravesically administered *cis*-platinum was given weekly × 6–8 doses and thereafter q2–4wks.

TABLE 2.—Phase II trials with *cis*-platinum at MSKCC

	Bladder cancer				Prostatic cancer		Penile cancer	Renal cancer
	cis-Platinum	*cis*-Platinum and CYC	*cis*-Platinum and Adriamycin	*cis*-Platinum, CYC, and Adriamycin	*cis*-Platinum	*cis*-Platinum, CYC, and Adriamycin	*cis*-Platinum	*cis*-Platinum
No. of patients—								
Entered	33	40	30	10	26	1	8	9
Not yet evaluated	4	0	3	4	0	0	0	0
With inadequate trial	1	5	1	0	1	0	2	0
With adequate trial	28	35	26	6	25	1	6	9
No. of responders (CR + PR)								
Total	10	15	14	3	3	0	3	0
With no prior chemotherapy/total patients	10/21	12/26	10/20	1/1	2/14	0/1	1/1	0/6
With prior chemotherapy/total patients	0/7	3/9	4/6	2/5	1/11	—	2/5	0/3
% of patients with—								
CR + PR	36	43	54	50	12	0	50	0
No prior chemotherapy	48	46	50	100	14	0	100	0
Prior chemotherapy	0	33	67	40	9	0	40	0
No prior radiotherapy	20	13	21	33	100	1	33	—
No. of nonresponders with—								
Minor response	4	2	1	1	0	—	0	1
Stabilization	0	0	0	0	1	—	0	1
Progression	14	18	11	2	21	—	3	7
Totals	18	20	12	3	22		3	9

by two or more perpendicular diameters. All patients had biopsy-proven urothelial cancer reviewed by the MSKCC Department of Pathology. Computerized transaxial tomographic (CTT) scans and roentgenograms were evaluated independently (R. C. Watson, MSKCC), and pelvic and abdominal lesions were generally examined by two observers (A. Yagoda and a member of the MSKCC Urology Service). Routine laboratory tests included an automated blood and platelet count, 12-channel screening profile, serum creatinine, 24-hour creatinine clearance, chest roentgenograms, iv pyelograms, bone surveys, and liver and bone scans. In selected cases, stereoscopic chest roentgenograms, pelvic CTT scans, and lymphangiograms were performed. Most patients had evaluations of the carcinoembryonic antigen (CEA), 5′-nucleotidase, and various coagulation factors. The serum acid phosphatase (SAP) sample was collected prior to rectal examination and was determined by a modification of the Bodansky method (6).

A dose of 1.25–1.6 mg/kg (50–70 mg/m^2) of *cis*-platinum was administered iv within 10 minutes following a 1-hour infusion of 500–1000 ml of 5% dextrose in water (3). Patients with borderline renal function (creatinine clearance < 60 ml/minute) received 12.5 g of mannitol iv at the time of *cis*-platinum administration and sufficient fluids to maintain a diuresis of 100–125 ml/hour for 6 hours. High-dose *cis*-platinum (3 mg/kg or 120 mg/m^2) was given with a mannitol infusion (7). All doses initially determined in milligrams per kilogram have been converted to milligrams per meter squared (table 1).

Since previous experience with *cis*-platinum in other solid tumors indicated rapid responses, an adequate trial was defined as one dose with or without hematologic depression and 3 weeks' survival, if active progression of disease was found. Otherwise, patients received *cis*-platinum until progression was noted, renal or auditory toxicity occurred, or nausea and vomiting resulted in the patient's refusal to continue therapy.

The following are the response criteria for all protocols. A CR was defined as a complete disappearance of all measurable, radiologic, and biochemical abnormalities. A partial response (PR) was defined as a $> 50\%$ decrease in the sum of the products of two or more perpendicular diameters of all lesions for > 1 month (for soft tissue lesions), a $> 75\%$ decrease by physical examination and/or a $> 50\%$ decrease by CTT scan in the sum of the products of two or more perpendicular diameters for > 1 month (for abdominal or pelvic masses), and a $> 50\%$ decrease by physical examination in the sum of all available measurements and a $> 50\%$ decrease in all biochemical abnormalities and filling defects on CTT scan for > 1 month (for hepatomegaly). (In addition, if pretreatment SAP, alkaline phosphatase, or CEA is abnormal, a $> 50\%$ decrease is required.) A minor response was defined as a 25%–50% decrease in tumor size or biochemical abnormalities for > 1 month or a $> 50\%$ decrease for < 1 month. Disease stabilization was defined as a $< 25\%$ decrease or increase in tumor size or biochemical abnormalities for > 3 months. Disease progression was defined as a $> 25\%$ increase in tumor size or biochemical abnormalities, or a mixed response. SAP or CEA levels (> 5.0 ng/ml in 70% of patients with bladder cancer) were included in the response criteria; PR required elevated pretreatment CEA or SAP levels to decrease $> 50\%$. However, a $> 50\%$ decrease in the CEA or SAP level alone was insufficient evidence for PR status. Mixed responses were evaluated separately but eventually were included in the progression category. Duration of response was measured from initiation of protocol until progression was noted. The Karnofsky performance score (PS) was used to evaluate subjective changes. Leukopenia was defined as a wbc count of $< 4500/\text{mm}^3$ and thrombocytopenia was defined as a platelet count of $< 175{,}000/\text{mm}^3$.

Patient Characteristics

The efficacy of antineoplastic drugs in the treatment of urothelial cancers is difficult to evaluate since these tumors frequently lack objectively measurable lesions such as lung, liver, skin, subcutaneous, or nodal metastases (8,9). Most patients with urothelial tumors entered in phase II trials have poorly measurable, so-called evaluable lesions, ie, intra-abdominal, rectal, or pelvic masses, lymphangitic spread of pulmonary metastases, or abnormal lymphangiograms, iv pyelograms, roentgenographic skeletal surveys, and/or radionuclide bone or liver scans. Additional parameters which are used to assess response include improvement of abnormal laboratory values, such as SAP, alkaline phosphatase, liver function tests, CEA, hemoglobin levels, and platelet counts, and improvement in such symptoms as pain intensity and analgesic requirements, PS, and weight loss. The potential inaccuracies in assessing objective changes in "evaluable" lesions, and in the subjective interpretation by both physicians and patients of favorable subjective benefit, can be misleading and may result in aberrant response rates (9).

Since the assessment of response in patients with urothelial cancer who have only "evaluable" parameters may be subjective, the true estimation of CR and PR rates as defined in the standard disease-oriented phase II trials (which normally demand a clear endpoint of response) is lacking. Therefore, only patients who have objectively "measurable" parameters are selected at MSKCC for disease-oriented phase II trials in urothelial cancers. Initially, the CTT scan was used to evaluate patients who had lymph node or pulmonary lesions and biopsy-proven pelvic metastases (4). Presently, lesions which can be measured bi-dimensionally by the CTT scan are accepted as "measurable" parameters. Approxi-

TABLE 3.—Results of phase II trials of *cis*-platinum in bladder cancer: measurable vs evaluable parameters

Parameters	*cis*-Platinum		*cis*-Platinum and CYC		*cis*-Platinum and Adriamycin		*cis*-Platinum, CYC, and Adriamycin	
	Total No. of patients	Patients with PR	Total No. of patients	Patients with PR	Total No. of patients	Patients with PR	Total No. of patients	Patients with PR
Measurable								
Lung, liver, skin, nodal, and subcutaneous lesions	22	8	25	13	24	13	5	2
Pelvic mass + CTT scan	1	0	5	3	1	1	1	1
Lymphangiograms	2	0	2	1	1	0	0	0
Evaluable								
Abdominal mass	2	2	3	0	0	0	0	0
Osseous lesions	1	0	0	0	0	0	0	0

mately 20 patients with "measurable" bladder cancer, eight with prostatic cancer, four with penile cancer, and 30 with renal cancer are seen at MSKCC annually. Patients who do not fulfill the selection criteria are offered other therapy. However, in spite of careful patient selection for "measurable" parameters, re-examination of roentgenograms and scans and the subsequent course and post-mortem findings occasionally resulted in patients being reclassified to the "evaluable" disease category. Data from these patients are included in the overall results but are presented separately (table 3).

There have been few published reports of the use of *cis*-platinum alone or in combination regimens in the treatment of urogenital cancers; consequently, drug-oriented phase I and II trials and disease-oriented phase II and III trials were extensively reviewed. In this paper the term *objective response* indicates only CR and PR status. Minor responses, disease stabilizations, mixed responses, and disease progressions are all included in the progression category.

BLADDER CANCER

The doses, schedules, and routes of administration of the four protocols used at MSKCC since 1975 are listed in table 1. *cis*-Platinum alone or in combination regimens was given to 113 patients who had nonresectable or metastatic tumors of the renal pelvis, ureter, bladder, or urethra. Eleven patients have not been evaluated, seven had inadequate studies, and 95 had adequate trials. Patient characteristics were similar for all protocols except for a higher percentage of patients who had had prior chemotherapy in protocol I compared to protocols II, III, and IV (tables 3 and 4).

Of 33 patients entered in protocol I (*cis*-platinum alone), 28 had an adequate trial. While the overall response rate was 36%, the PR rate in previously untreated patients was 48% (ten of 21 patients; *see* table 2). The median duration of response was 5 months, with the majority of remissions unmaintained. The longest unmaintained remission was 9 months. No patient was classified in CR status because of the inability to accurately evaluate remission status in intra-abdominal sites (3).

Protocol II (*cis*-platinum and cyclophosphamide [CYC]) was given to 40 patients, and 35 had an adequate trial. The overall response rate was 43% (15 of 35 patients) which was similar to that obtained with *cis*-platinum used singly. The response rate in patients who had had no prior chemotherapy was 46% (12 of 26 patients) vs 33% (three of nine patients) in previously treated patients (table 2). No difference in response rates occurred when CYC was given at a dose of 250–750 mg/m^2 (4). Toxicity resulted in cessation or temporary delay in therapy because of vomiting and nausea in 14 patients, myelosuppression in seven, hematuria in five, and mild nephrotoxicity in 19. Renal function generally improved to pretreatment status within 2–8 weeks. Myelosuppression correlated with the dose of CYC and the extent of prior irradiation. Although the combination of *cis*-platinum and CYC increased the median duration of response to 7 months, survival was similar to that obtained with *cis*-platinum alone (4).

Protocol III (*cis*-platinum and Adriamycin) has been administered to 30 patients and 26 are evaluable. The overall response rate is 54% (14 of 26 patients) with a median duration of 3+ months. Two patients appear to have achieved CRs. The response rate is 50% (ten of 20 patients) in previously untreated patients and 67% (four of six patients) in previously treated patients (table 2). *cis*-Platinum and Adriamycin produced more myelosuppression than the combination of *cis*-platinum and CYC but less nausea and vomiting. It is too early to evaluate protocol IV which combines *cis*-platinum, CYC, and Adriamycin.

Sixteen patients with low-stage bladder tumors, ie, carcinoma in situ, papillomas, and stage A epidermoid

TABLE 4.—Phase II trials in adequately treated patients with urothelial cancers

Patient characteristics	*cis*-Platinum	*cis*-Platinum and CYC	*cis*-Platinum and Adriamycin	*cis*-Platinum, CYC, and Adriamycin
Median age in yrs (range)	59 (39–73)	60 (30–79)	64 (35–77)	61 (47–71)
% Males	86	63	73	100
Median PS (range)	75 (30–90)	80 (30–90)	80 (50–100)	80 (50–90)
% of patients with—				
Tumor grade III/IV	57	51	54	100
Prior urinary diversion	54	54	19	33
Prior irradiation	79	85	77	83
2+ ports	25	12	15	33
Prior chemotherapy	32	26	23	17
Median time in mos from—				
Diagnosis to protocol (range)	16 (0–72)	16 (3–135)	19 (2–149)	28 (9–121)
Protocol to followup (range)	4 (1+–34)	6 (1.2–24+)	6+ (1.5–15+)	2+ (1+ –4+)

carcinomas, received iv *cis*-platinum (table 1). In addition, 22 patients with similar lesions were given 75–150 mg of *cis*-platinum in a concentration of 1 mg/ml intravesically for 1–2 hours every week for six to eight doses and thereafter every 2–4 weeks (10). Intravesical administration of *cis*-platinum was well-tolerated without evidence of significant systemic absorption, except for one patient who had a pruritic rash after four doses of *cis*-platinum. The criteria for response used at MSKCC for intravesical therapy were composed of a four-component system (O = papillomas, P = urine cytology, E = epidermoid carcinoma, and C = carcinoma in situ) which recognized only CR of all lesions as indicative of a therapeutically beneficial remission. While tumor regression and absence of tumor recurrence were frequently documented, except for two patients, the majority of responses were incomplete and relapse occurred in 6 months.

Literature Review

cis-Platinum used singly has been given to 43 patients in four separate trials. Thirty-nine patients had adequate trials and 19 (49%) achieved CR or PR status. Merrin (11) described one CR and eight PRs in 19 patients when 1 mg/kg of *cis*-platinum was administered by a 6–8-hour infusion every week for 6 consecutive weeks followed by an every-3-week maintenance schedule. Two additional patients obtained minor responses. Soloway (12) noted one CR and five PRs among ten patients given 1.6 mg/kg of *cis*-platinum every 3 weeks. The median duration of response was 4.3 months (range, 2–6+ months). Rossof et al (13) obtained three PRs in nine patients who received 75 mg/m^2 of *cis*-platinum every 3 weeks. Wallace and Higby (1) described a CR of > 1 year in a patient who received two doses of 100 mg/m^2 of *cis*-platinum. Although no maintenance therapy was given, the CR persisted for 4 years (11).

A combination of the data from the present study with those of the literature shows that among 76 patients receiving *cis*-platinum, 67 (88%) had adequate trials and 30 (45%) achieved CR or PR. Minor responses were seen in six additional patients. The average duration of response was 4–5 months (range, 2–10 months).

No remissions were seen in two patients treated with the combination of *cis*-platinum and Adriamycin. However, Williams et al (14) recently reported PRs of 1–9 months' duration in ten of 16 adequately treated patients with a combination of *cis*-platinum, Adriamycin, and 5-fluorouracil (5-FU). A study by the European Organization for Research on Treatment of Cancer (15) with Adriamycin and 5-FU in bladder cancer noted responses in 40% of 52 patients (four CRs and 17 PRs) with an average duration of 9 months (range, 2–16 months).

The combination of *cis*-platinum (100 mg/m^2 on Day 1), Adriamycin (50 mg/m^2 on Day 2), and CYC (650 mg/m^2 on Day 1) was reported to produce an 83% response rate in 12 adequately treated patients (16). Although the median time to response was 3.5 weeks, the duration of response was not stated. Troner and Hemstreet (17) who used the same drug regimen but with different doses (40 mg/m^2 of *cis*-platinum, 50 mg/m^2 of Adriamycin, and 500 mg/m^2 of CYC) described one CR and three PRs among nine adequately treated patients. The duration of response was 3+ to 9 months. Of note, the response rate

of 44% described by Troner and Hemstreet (17) is similar to that of *cis*-platinum used singly.

PROSTATIC CANCER

The characteristics of 25 adequately treated patients are outlined in table 5. Although the median age of the patients was 65 years, 28% were ≤ 60 years. Twenty-two of 25 patients had had some form of previous endocrine therapy and 18 had achieved remission. Eleven patients (44%) had had prior chemotherapy alone or in combination regimens which consisted of Adriamycin, 5-FU, hydroxyurea, and alkylating agents. Sixteen patients had had prior irradiation; in 11 of these patients (44%) it involved significant bone marrow areas.

Twenty patients had "indicator" lesions: 12 had palpable lymph nodes, or penile, breast, and other cutaneous and subcutaneous masses, including five with hepatomegaly and three with pulmonary metastases; six had pulmonary or extrapleural lesions, including four with hepatomegaly; and two had malignant hepatomegaly, including one who was previously untreated and had an abnormal lymphangiogram and a large prostatic mass. Five patients were reclassified as having "evaluable" disease: two had large rectal and prostatic masses evaluable by CTT scan (including one with an abnormal lymphangiogram), and three had elevated SAP.

Sixty percent of the patients (14 of 24) had an elevated SAP, 68% had a hemoglobin value of < 12.4 g/100 ml, and 64% (nine of 14) had a CEA of > 5.0 ng/ml. Twenty-one of 24 patients had abnormal bone scans and 20 of 23 patients had abnormal skeletal surveys.

Results

A total of 69 doses (median, three) were administered to 25 adequately treated patients; seven patients (28%) had one dose, seven (28%) had two doses, and 11 (44%) had three to eight doses. Nausea and vomiting occurred in 18 patients (72%) and was severe in seven (28%). Nine patients had leukopenia with a median wbc count of 2.6 × 10^3/ml (range, 0.8–4.4 × 10^3/ml). Nine patients (36%) showed unexplained transient BUN (median, 32 mg/100 ml) or creatinine elevations which generally returned to pretreatment levels within 4–8 weeks after cessation of therapy.

PR status was achieved in three patients (12%) for 3, 2.5, and 2 months, respectively (table 2). One patient (4%) had disease stabilization for 8 months. Eight additional patients (32%) had dramatic relief of bone pain beginning 1–4 days after *cis*-platinum administration, yet most of these patients exhibited definite progression of measurable parameters. If pain relief, osseous lesions, and SAP and alkaline phosphatase levels were the only measurable parameters, 48% of patients would have been categorized as achieving disease stabilization.

TABLE 5.—*cis*-Platinum in the treatment of prostatic carcinoma: patient characteristics

Patient characteristics	No. of patients with— Progression (total: 21 patients)	PR-stabilization (total: 4 patients)
Age (yrs)		
51–60	6	1
61–70	12	2
71+	3	1
(median)	(66)	(65)
PS		
≥ 80	5	2
60–70	8	1
40–50	7	1
≤ 30	1	0
Prior chemotherapy	9	2
Prior hormonal therapy	18	4
Median time (mos) from—		
Diagnosis to hormone	4	1
Hormone to protocol	21	
Prior orchiectomy	12	4
Median time (mos) from—		
Diagnosis to orchiectomy	1	13
Orchiectomy to protocol	30	27
Prior radiation therapy	16	4
Median time (mos) from—		
Radiation to protocol	2	1
Increased SAP (> 0.8 unit/dl)	12*	2
Increased CEA (> 5.0 ng/ml)	8†	1‡
Hemoglobin (g/100 ml)		
≥ 10.5	15	4
≤ 10.4	6	0
Platelet count (< 175,000 cells/mm³)	5	0
Median time from—		
Symptoms to diagnosis (mos)	12§	5
Diagnosis to metastasis (mos)	1	30
Diagnosis to protocol (mos)	26	48
Protocol to survival (wks)	20	53

*20 evaluable patients.
†11 evaluable patients.
‡2 evaluable patients.
§19 evaluable patients.

There was no significant difference between the PR-stabilization group and the progression group with respect to age, prior chemotherapy, limited or extensive irradiation, and levels of SAP and alkaline phosphatase. However, significance was found for PR-stabilization patients, all of whom had had an orchiectomy, a hemoglobin value of 10.5 g/100 ml prior to *cis*-platinum administration, and a longer interval from diagnosis to metastases

(30 months versus 1 month for progression) and from diagnosis to protocol (48 months versus 26 months for progression). The prognostic significance of various parameters was also examined, and of particular interest was the longer survival and significantly longer interval from diagnosis to protocol for the PR-stabilization group versus the progression group (table 5). Although the duration of response was only 2 or 3 months, the survival difference for responders versus nonresponders was considerable, 53 versus 20 weeks, suggesting a slower growth pattern of disease in the responders.

Literature Review

All published phase I and II trials of *cis*-platinum used alone or in combination drug regimens were reviewed. Forty-three patients were treated with *cis*-platinum alone and 34 of these were considered to have adequate trials. Hill et al (18) and Rosoff et al (13) noted one PR in 13 adequately treated patients. In contrast, Merrin (19) described nine PRs (43%) which persisted for 3–14 months in 21 patients given *cis*-platinum at a dose of 1 mg/kg every week in a 6-hour infusion for six consecutive doses followed by an every-3-week maintenance schedule. The average total dose of *cis*-platinum was 527 mg/patient which was higher than the total dose used in the present study.

Combining the data of the present series and all published reports of *cis*-platinum used singly results in an overall PR rate of 22% in 59 adequately treated patients. The PR-stabilization rate is 36%.

A dose of 50 mg/m^2 of *cis*-platinum has been used in combination with 50–60 mg/m^2 of Adriamycin every 3 weeks. Perloff et al (20), Vogl et al (21), and Ohnuma et al (22) reported nine PRs (53%) in 17 of 21 evaluable patients. The median duration of response was 5 months. Mills et al (23) described two of two patients who achieved PR status for 5 and 8 months, respectively, but responses consisted of "control of pain and laboratory abnormalities." The only other report of *cis*-platinum in prostatic cancer is that of Samson et al (24) who found no response in a patient given *cis*-platinum, bleomycin, and vinblastine.

PENILE CANCER

Eight patients with penile cancer received *cis*-platinum: five at a dose of 120 mg/m^2 and three at a dose of 60 mg/m^2. The median age was 57 years (range, 45–67 years) and the median PS was 65 (range, 40–90). Three patients had had groin dissections, four had had prior irradiation, and seven had had prior chemotherapy (bleomycin or methotrexate). Measurable parameters included palpable lymph node (eight patients), pulmonary (three), and skin (two) metastases.

Three of six adequately treated patients (50%) obtained PRs (table 2) for 1+, 2+, and 7 months. Responses were rapid, occurring within 2–21 days, and were accompanied by symptomatic improvement and an increase in PS. A recent review of chemotherapy trials in penile cancer indicates that *cis*-platinum is one of the more active agents in the treatment of metastatic squamous cell carcinoma of the penis.[5]

RENAL CANCER

Nine patients with hypernephroma received *cis*-platinum: six at a dose of 100–200 mg/m^2 and three at a dose of 80 mg/m^2. The median age was 59 years (range, 26–77 years) and the median PS was 70 (range, 30–90). Four of nine patients had had prior irradiation and three of nine had had prior chemotherapy. Eight patients had "measurable" disease: six had lung lesions and two had malignant hepatomegaly. There were no responses (table 2), and a review of the literature (13,25) has revealed only one PR (2%) in 49 patients.

DISCUSSION

The results of all phase II trials at MSKCC are summarized in table 2. *cis*-Platinum does produce a significant response rate (CR or PR) in patients with advanced urinary tract cancer. Responses are generally rapid and most patients achieve PR status within one or two doses, while additional doses do not induce CR; cessation of therapy generally results in relapse in 6–8 weeks. Occasionally, patients will have an unmaintained remission for 9–12 months. While the median duration of response is only 5–7 months, responders live for a median of 11 months (range, 2–15+ months) and nonresponders live for a median of 4 months (range, 1–9 months) ($P < 0.01$) (4). Results from a literature review, coupled with results from the present study, indicate an overall response rate of 45% regardless of the schedule or dose used. The 95% confidence limit is 12%–57%.

Protocols II, III, and IV, which used combination regimens, were no more effective than *cis*-platinum alone. While some reports have indicated improvement in remission rates when *cis*-platinum is combined with other agents, differences in case selection and response criteria may account for apparent increases in the number of remissions rather than additive or synergistic effects of *cis*-platinum combination regimens. Whether combination regimens are really superior to *cis*-platinum alone is unanswered and can only be determined in prospective randomized trials with a large number of patients who have objectively "measurable" parameters.

[5]Sklaroff RB, and Yagoda A. *cis*-Diamminedichloride platinum II (DDP) in the treatment of penile cancer. Cancer. In press.

The efficacy of iv or intravesical chemotherapy for established or recurrent low-stage bladder tumors frequently reflects the conviction of the treating physician. Intravesically administered agents may destroy established tumors or micrometastases but premalignant "new" lesions which may only be visualized by cystoscopy 3–12 months after therapy may be unaffected. In addition, persistently positive urine cytology studies could indicate treatment failure or evidence of residual disease secondary to a carcinogenic-induced field effect in the ureter, urethra, or prostatic ducts. Thus, the potential use of *cis*-platinum, or, in fact, any intravesical therapy for low-stage bladder lesions needs further clarification.

The presently available data suggest that *cis*-platinum is at best an induction agent and additional drugs are needed for consolidation and maintenance regimens. With the 45% remission rate attainable with *cis*-platinum alone or in combination regimens, adjuvant chemotherapy trials should be initiated in a controlled prospective fashion in patients who have a high risk of bladder cancer recurrence after a potentially curative procedure.

cis-Platinum administered in the dose and schedule used in this protocol was not an active agent in the treatment of adenocarcinoma of the prostate. The higher remission rate found by Merrin (19) may reflect not only dose and schedule differences, but different response criteria, definitions of measurable disease, and patient characteristics. Possible therapeutic benefit of *cis*-platinum in this tumor will need further clinical trials.

While *cis*-platinum has shown no antitumor effect in hypernephroma, responses have been seen in a small number of cases of penile cancer. Further trials with *cis*-platinum in the treatment of penile cancer are needed and future protocols should consider a combination of *cis*-platinum and bleomycin.

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Advanced Bladder Cancer: Therapy With *cis*-Dichlorodiammineplatinum(II), Adriamycin, and 5-Fluorouracil [1,2]

Stephen D. Williams,* John P. Donohue, and Lawrence H. Einhorn [3]

SUMMARY

Nineteen patients with unresectable or metastatic urothelial cancer were treated with the combination of *cis*-dichlorodiammineplatinum(II), Adriamycin, and 5-fluorouracil. One patient died within 2 weeks of therapy and one patient had no measurable disease, leaving 18 patients evaluable for survival and toxicity and 17 patients evaluable for response. Eleven patients (65%) achieved a partial remission with attendant clinical improvement, with a median duration of 25 weeks. All nonresponders have died, with a median survival of 17 weeks. The median survival of responding patients was 40 weeks, with two patients alive at 33+ and 63+ weeks. Of particular interest are three patients who had resections of residual pelvic disease after significant regression with chemotherapy. One of these patients remains disease-free at 63+ weeks. Toxicity was significant but generally manageable.

[Cancer Treat Rep 63:1573–1576, 1979]

Chemotherapy for advanced bladder cancer in general has been discouraging. Of the standard agents, Adriamycin, 5-fluorouracil (5-FU), and mitomycin C have been the most extensively evaluated, yielding response rates of 25%–33% (1). Similarly, methotrexate produced an overall response rate of 38%, although rigorous response criteria were not used (2). Cyclophosphamide was initially reported to have a response rate of approximately 50%, either alone or in combination with Adriamycin (3). However, more recent experience, using strict response criteria, has been considerably less favorable. Yagoda et al (4) found only a 14% response rate to Adriamycin in 35 evaluable patients. The same investigators found only a 17% response rate in 18 patients with urothelial cancer treated aggressively with Adriamycin plus cyclophosphamide at a cost of considerable hematologic toxicity (5).

More recently, the investigational agent, *cis*-dichlorodiammineplatinum(II) (*cis*-platinum), was shown to have activity in a variety of urinary tract malignancies. Its major role appears to be in combination regimens for testicular cancer (6). In addition, Yagoda et al reported a 35% response rate with *cis*-platinum in bladder cancer; furthermore, eight of 14 patients with no prior chemotherapy had an objective response (7). The dose-limiting toxicity of *cis*-platinum is renal damage and it has a relative lack of myelosuppression, making it an attractive agent for combination chemotherapy (8). There is evidence in animal tumor systems of synergism with the combination of Adriamycin plus *cis*-platinum (9); in addition, *cis*-platinum is suitable for repeated administration (10). *cis*-Platinum plus Adriamycin can be given to patients with acceptable toxicity (9,11), even in the face of moderate renal failure (12).

The combination of Adriamycin plus 5-FU has been evaluated, producing seven partial responses among 13 patients in one series (13), three complete and four partial responses among 20 evaluable patients in a second series (14), and four complete and 17 partial responses among 52 patients in a third series (15). This combination has been generally well-tolerated.

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For these reasons, the combination of *cis*-platinum, Adriamycin, and 5-FU was chosen for evaluation in patients with advanced bladder cancer. Cyclophosphamide was not used because of concern that prior radiotherapy would increase the likelihood of hemorrhagic cystitis in patients with intact bladders (16).

PATIENTS AND METHODS

After informed consent was obtained, 19 patients with advanced urothelial cancer were treated. Their pretreatment characteristics are shown in table 1. One patient probably had a ureteral tumor; the origin of the remaining tumors was the bladder. Seventeen patients had transitional cell carcinoma, with one each having squamous carcinoma and adenocarcinoma. Nine patients had had prior radiotherapy to fields encompassing major bone marrow-bearing areas. Six had had previous "curative" surgery. None had had prior chemotherapy. Patients with significant organic heart disease or serum creatinine levels of > 2.0 mg/100 ml not due to obstructive uropathy were excluded.

Pretreatment evaluation included history and physical examination, cbc, liver function studies, routine radiographs, electrocardiogram, creatinine clearance, audiometry, bone and liver scans, abdominal ultrasound, and, on occasion, abdominal computerized tomography.

The treatment regimen is shown in figure 1. The initial doses of 5-FU and Adriamycin were reduced by 20% for patients with prior pelvic radiotherapy or for patients who were > 65 years old. The Adriamycin dose was reduced by 50% if the pretreatment bilirubin level was > 2.0 mg/100 ml. Diuretics and mannitol were not used, but during induction therapy all patients were given normal saline or half-normal saline in 5% dextrose at a rate of 100 ml/hour. During maintenance therapy, 500–1000 ml of iv fluids were given on the day of chemotherapy. Likewise, aminoglycoside antibiotics were avoided, since these, in our experience, may potentiate *cis*-platinum-induced nephrotoxicity (6).

TABLE 1.—Patient characteristics

Total No. of patients	19 (15 males, 4 females)
No. of evaluable patients	18
No. of patients with measurable disease	17
Median age in yrs	63 (range, 51–76)
No. of patients with prior radiotherapy	9
No. of patients with prior definitive surgery	6
Sites of involvement	
Local only	4
Local + distant	8
Distant only	7

Induction therapy

cis-Platinum, 20 mg/m^2 iv over 15-30 mins, Days 1-5
Adriamycin, 50 mg/m^2 by iv push, Day 1
5-FU, 500 mg/m^2 by iv push, Day 1

Given every 4 wks for 3 courses with continuous iv hydration (see text)

Maintenance therapy

cis-Platinum, 50-75 mg/m^2 iv over 2 hrs
Adriamycin, 50 mg/m^2 by iv push
5-FU, 500 mg/m^2 by iv push

Given every 4 wks with iv hydration (see text)

FIGURE 1.—Treatment regimen.

Blood cell counts were obtained weekly during induction therapy and subsequently before each maintenance treatment. Liver function studies, serum creatinine, and creatinine clearance were obtained prior to each course. Radiographic procedures and scans that were initially abnormal were repeated during the third induction course and were subsequently repeated as indicated. Tumor measurements and physical examination were done prior to each course. An electrocardiogram was done before each dose of Adriamycin after a total cumulative dose of 300 mg/m^2 had been reached.

A partial remission was defined as at least a 50% reduction in measurable lesions with no subjective deterioration. Progression was defined as a > 25% increase in such lesions.

Therapy was continued until progression was noted or toxicity precluded further treatment. All treatment was stopped if the serum creatinine rose to > 2.5 mg/100 ml.

Resection of residual pelvic disease was accomplished by total cystectomy, pelvic lymphadenectomy, and urinary diversion by ileal conduit.

RESULTS

Toxicity

Toxic manifestations are shown in table 2. Therapy was relatively well-tolerated. Alopecia was universal, as was moderate to severe nausea and vomiting, causing one patient to refuse further therapy after two courses. One patient was successfully treated for granulocytopenic sepsis at a time when his disease was rapidly progressing. During this episode, his creatinine rose to 6.7 mg/100 ml after receiving gentamicin. Only one other patient at any time had a creatinine of > 2.5 mg/100 ml; the *cis*-platinum nephrotoxicity was quite acceptable. In general, there was little or no change in serum creatinine during the three induction courses. Subsequently, it

TABLE 2.—Toxicity

	No. of patients (%)
Nausea and vomiting	18 (100)
Alopecia	18 (100)
Symptomatic hearing loss	3 (16.7)
Cardiotoxicity	0
Thrombocytopenic bleeding	0
Anemia (requiring transfusion)	5 (27.8)
Nephrotoxicity (creatinine, > 2.5 mg/100 ml)	2 (11.1)
Granulocytopenic sepsis	1 (5.6)

slowly rose during the maintenance phase of therapy. The median increase in serum creatinine from baseline to maximum was 0.4 mg/100 ml.

Ototoxicity was troublesome for three patients, with one of these patients experiencing near-total hearing loss.

There were no drug-related deaths and the three patients who had cystectomy had no operative morbidity or mortality.

Therapeutic Response

One patient died from massive tumor within 10 days of initiation of therapy, and one patient had no measurable disease. This patient, however, had subjective improvement with significant decrease in bone pain.

Of fully evaluable patients, 11 of 17 (65%) obtained a partial remission and another two had stabilization of disease for at least 2 months. Remissions were associated with subjective improvement in all symptomatic patients, which at times was quite striking. Median duration of remission was 25 weeks (range, 12–59+); only one patient is in continuous remission. There were no drug-induced complete remissions. The number of treatment courses per patient ranged from one to nine, with a median of 4.5.

All nonresponding and stable patients are dead, with a median survival of 17 weeks. The survival of responding patients ranged from 20 to 78 weeks (median, 40), and only two are alive at 33+ and 63+ weeks.

Bone, nodal, and lung metastases as well as pelvic disease seemed to respond equally well. Only three patients had liver metastases, however, and none of these clearly responded.

Of particular interest are three patients who had resections of residual pelvic tumor. Two of these patients had extra-pelvic metastases that totally disappeared; both developed rapidly progressive disease and died at 29 and 39 weeks. The third patient had a large pelvic mass that was initially unresectable and was causing severe symptoms. He dramatically improved with chemotherapy and the mass became resectable. He is now disease-free at 63+ weeks.

DISCUSSION

The lack of effectiveness of standard chemotherapeutic agents and the impressive single-agent activity of *cis*-platinum have caused considerable interest in the use of *cis*-platinum-based combination regimens in bladder cancer. Yagoda et al (17) evaluated the combination of *cis*-platinum plus cyclophosphamide, which yielded a 47% response rate with a median duration of 7 months. In another study, nine of ten patients responded to the combination of *cis*-platinum, cyclophosphamide, and Adriamycin (18). Data on remission duration and survival are not yet available from this study. Results in all of these series are superior to those obtained with previous single agents and combinations.

It is discouraging that the response duration was relatively short in our study. However, we were impressed by the rapidity of response and symptomatic improvement of many of our patients and the general quality of remissions. The potential toxicity of this regimen is, however, fairly formidable, requiring close medical supervision, and its use probably should be reserved for symptomatic patients. Additional active drugs are urgently needed.

The overall impact of *cis*-platinum combinations on survival remains speculative but is probably modest. In addition, the use of combinations as opposed to the use of *cis*-platinum alone is open to question, and this should be evaluated in a random prospective fashion.

However, there are two additional aspects of this therapy that are extremely exciting. Carefully selected patients may be rendered disease-free by resection of residual pelvic tumor after responding favorably to chemotherapy.

Our two patients who had extra-pelvic metastases that regressed completely did very poorly and cystectomy cannot be recommended in this situation. However, the patient with pelvic disease only who had resectable disease after chemotherapy remains disease-free and, in fact, is the only long-term survivor. Thus, we feel that an attempt at surgical extirpation of residual disease should be made in patients in this situation. Optimistically, a number of such patients may experience long-term survival.

Likewise, there is a definable group of patients with invasive but resectable disease who nonetheless have a very poor prognosis. *cis*-Platinum or a *cis*-platinum-based combination would appear to have enough activity to warrant investigation in a random prospective fashion as an adjuvant to preoperative radiation therapy and cystectomy. Anecdotally, an additional patient (who re-

ceived 10 months of *cis*-platinum-based combination chemotherapy) not included in this study remains disease-free 23 months after resection of a metastatic supraclavicular lymph node which was his only evident disease.

cis-Platinum-based chemotherapy represents a significant improvement in the therapy for metastatic bladder cancer. However, its major value may be as adjuvant therapy in patients with localized invasive disease.

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Phase II Trial of *cis*-Dichlorodiammineplatinum(II) in Cancer of the Bilharzial Bladder[1]

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A variety of agents have been used in phase II trials in cancer of the bilharzial bladder in Egypt. Only hexamethylmelamine has demonstrated clinical benefit (1–3). In a continued search for active drugs a trial of *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) was undertaken based upon responses in transitional cell carcinoma of the bladder in the US (4).

PATIENTS AND METHODS

All patients were evaluated and treated at the National Cancer Institute, Cairo, Egypt, between June 1977 and August 1978. Only patients with UICC (International Union Against Cancer) stage III or IV (inoperable) and measurable tumors were treated. Furthermore, all patients had to have bilaterally functioning kidneys demonstrated by intravenous pyelogram, a serum creatinine level of < 1.5 mg/100 ml, and a BUN level of < 40 mg/100 ml to be eligible for *cis*-platinum treatment. The diagnosis was established histologically and/or cytologically. No patient had received prior chemotherapy or radiotherapy.

cis-Platinum was administered in 8-hour iv infusions containing 3 liters of normal saline. Diuresis of at least 150–200 ml/hour was induced by furosemide and mannitol during the infusion and for 24 hours thereafter. Courses were repeated at 3-week intervals. The starting dose for the first trial (18 patients) was 30 mg/m^2, with a 10-mg/m^2 escalation for the second dose if no adverse hematologic or renal toxicity was encountered. The dose for all patients in the second trial (20 patients) was 50 mg/m^2 with subsequent dose escalation to 60 mg/m^2 if the first dose was well-tolerated. Responses were judged by standard phase II criteria (1–3).

RESULTS

Table 1 shows the response rates according to histology and drug dose. One complete and four partial responses were observed among 38 evaluable patients. The complete responder was a 59-year-old male with a large pelvi-abdominal mass with invasion of the abdominal wall. The diagnosis was established cytologically. Complete regression of the tumor occurred after two doses of *cis*-platinum, and urinary cytology became normal. The patient remains in remission at 18+ months and *cis*-platinum has been continued. Partial responses lasted 2–4 months. Four patients had stable disease and the remaining patients had progressive tumor growth. Seventeen of the latter group received only one course of *cis*-platinum because of advancing renal dysfunction, tumor progression, or other tumor-related complications.

Toxic effects were minimal. Only four patients experienced nausea and vomiting. Seven patients developed uremia during treatment, but the cause could not be determined to be drug-related because of severe obstructive urinary tract disease. No acute drug-related deaths were encountered and no clinically significant hematologic, neurologic, or auditory toxicity was noted.

DISCUSSION

There were eight partial responses among 14 patients (57%) in a phase II clinical trial of transitional cell bladder cancer using *cis*-platinum (4). The present study was conducted in patients with predominantly squamous cell bladder carcinoma. Although *cis*-platinum is active in squamous cell cancers of the head and neck (5), the results of this phase II trial indicate minimal antitumor effect. One patient did achieve a complete regression of tumor following two courses of drug, and four patients fulfilled criteria for a partial response. The responses, however, were relatively brief. It did not appear from this study that dose escalation improved the response rate (three responses among 18 patients at 30 mg/m^2 and two responses among 20 patients at 50 mg/m^2). The excellent drug tolerance, particularly the lack of nausea and vomiting, may be related to a prolonged infusion, the low dose of *cis*-platinum, and the induction of diuresis.

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TABLE 1.—Results of 2 sequential phase II trials of *cis*-platinum

Histologic type	No. of patients	No. of responses				No. of courses			Toxicity	
		Complete	Partial	Stable disease	Progressive disease	1	2	3+	Gastro-intestinal	Renal
cis-Platinum (30 mg/m^2)										
Squamous cell	17	1	2	3	11	9	2	6	2	4
Transitional cell	1	0	0	0	1	0	1	0	0	0
cis-Platinum (50 mg/m^2)										
Squamous cell	16	0	1	1	14	6	3	7	2	3
Transitional cell	4	0	1	0	3	2	2	0	0	0
Totals	38	1	4	4	29	17	8	13	4	7

Statistical analysis of these results (five responses among 38 patients) indicates that *cis*-platinum is active at a rate of 10% but with a false-positive probability of 50% (6). A third trial was initiated using *cis*-platinum at a dose of 20 mg/m^2 in daily 8-hour infusions for 5 days. Results of this trial reveal no responses in 24 patients. We conclude that *cis*-platinum is relatively inactive in this disease.

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Treatment of Genitourinary Tumors With *cis*-Dichlorodiammineplatinum(II): Experience in 250 Patients

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SUMMARY

Two hundred and fifty patients with genitourinary tumors were treated with *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) alone or in combination with other drugs or hormones (76 patients with stage III nonseminomatous testicular tumors, five with stage III seminomas, 86 with stage D prostatic carcinomas, 51 with bladder carcinomas, ten with renal cell carcinomas, three with rhabdomyosarcomas, three with penile carcinomas, and 15 with locally invasive bladder carcinoma treated by intravesical instillation of *cis*-platinum). Sixty-six patients with nonseminomatous testicular tumors who were treated with *cis*-platinum, bleomycin, vincristine, prednisone, and actinomycin D in combination with reductive surgery showed an initial complete remission rate of 81.8% (54 patients); 41 patients (62.1%) have remained in complete clinical remission for 6–40 months. Patients with nonseminomatous testis tumors treated with *cis*-platinum alone showed complete clinical remissions which lasted from 4 to 8 months, with an average of 5.5 months. A complete clinical remission was seen in four of five seminoma patients. In a group of hormonal-resistant patients with carcinoma of the prostate, a partial objective response was seen in 17 of 54 (31.4%). Thirty-two other patients with previously untreated carcinoma of the prostate were treated with orchiectomy, estrogen therapy, and *cis*-platinum. A partial objective response was seen in 13 patients (40.6%). Nineteen of 51 patients (37.2%) with bladder carcinoma showed a partial objective response. No response was seen in ten patients with renal cell carcinoma. A partial objective response was seen in two of three patients with rhabdomyosarcomas. A partial objective response was seen in eight of 15 patients with locally invasive carcinoma of the bladder treated with intravesical instillation. *cis*-Platinum appears to be active in testis tumors, adenocarcinoma of the prostate, and bladder carcinoma. It is not effective in renal cell carcinoma.

[Cancer Treat Rep 63:1579–1584, 1979]

The wide spectrum of anticancer activity of *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) (1) has prompted us to determine its efficacy in genitourinary tumors. Since 1974, we have administered *cis*-platinum alone or in combination with hormones or other drugs to 250 patients. Our experience is reported herein.

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MATERIALS AND METHODS

Two hundred and fifty patients with genitourinary tumors were entered in this study. There were 76 patients with stage III nonseminomatous testicular tumors, five with stage III seminomas, 86 with stage D prostatic carcinomas, 51 with stage D bladder carcinomas, ten with stage IV renal cell carcinomas, three with advanced rhabdomyosarcomas, three with metastatic penile carcinomas, and one with adrenal carcinoma. There were also 15 patients with locally invasive bladder carcinomas treated with intravesical instillation of *cis*-platinum.

Sixty-six of the patients with nonseminomatous testic-

Remission Induction Therapy (6 wks)
Bleomycin (30 units/day) infused in 5 hrs on wks 1 and 3
cis-Platinum (1 mg/kg) and vincristine (2 mg) on wks 1-6
Prednisone (10 mg bid) on wks 1-4

Consolidation Therapy (9 wks)
Actinomycin D (0.5 mg/day x 5 days) on wks 7 and 13
cis-Platinum (1 mg/kg) and vincristine (2 mg) on wks 7, 10, and 13

Maintenance Therapy (2 yrs)
Actinomycin D (0.5 mg/day x 5 days) q6wks
cis-Platinum (1 mg/kg) q3wks

FIGURE 1.—Chemotherapy schedule for advanced testicular tumors.

ular tumors were treated with a combination of *cis*-platinum, bleomycin, prednisone, vincristine, and actinomycin D (fig 1). These patients initially received chemotherapy and were completely re-evaluated after 2 months of treatment. Those who achieved a complete clinical remission continued with chemotherapy for a period of 2 years. Patients who achieved a partial clinical remission only had excisions of the residual tumor and continued to receive chemotherapy for a total period of 2 years. The seminoma patients also received the same drug combination. The other ten patients with nonseminomatous testes tumors had been previously treated with other drugs and failed to respond to therapy; they received *cis*-platinum alone.

Fifty-four patients in the stage D prostatic carcinoma group failed to respond to previous therapy and were estrogen-resistant; they received *cis*-platinum alone. The 32 remaining patients in this group had not been previously treated and received *cis*-platinum in combination with diethylstilbestrol (1 mg/day) and orchiectomy (androgen suppression).

Twenty patients in the stage D bladder carcinoma group had received radical cystectomy and ileal loop diversion before starting treatment; the remaining 31 patients still had their bladders intact. All of these patients received *cis*-platinum alone.

Seven of the ten patients with advanced renal cell carcinoma had undergone a previous excision of their primary tumor in the kidney. The three patients with rhabdomyosarcomas had failed to respond to treatment with previous chemotherapy. The three patients with penile carcinoma had not been previously treated, and the patient with adrenal carcinoma had failed to respond to previous treatment with *ortho-para*-dichlorodiphenoldichloroethane.

Patients with bladder carcinoma, renal cell carcinoma, rhabdomyosarcoma, penile carcinoma, and adrenal carcinoma received *cis*-platinum alone.

All of the patients in this study were evaluated by physical examination, chest roentgenogram, chest tomography, metastatic bone survey, aortic angiography, vena cavography (testes tumors), bone, liver, and brain scans, iv urography, pedal lymphangiography, tissue biopsies, endoscopy, routine biochemical and hematologic studies, and biologic markers for testicular tumors (α-fetoprotein and β subunit of human chorionic gonadotropin).

METHOD OF *CIS*-PLATINUM ADMINISTRATION

The patients in this study were treated with 1 mg/kg of *cis*-platinum once weekly for the first 6 weeks and every 3 weeks thereafter. The *cis*-platinum was mixed with 37.5 g of mannitol and 40 mEq of potassium chloride mixed in 2000 ml of 5% dextrose in ⅓ normal saline solution. The *cis*-platinum was administered in a slow iv infusion lasting 6–8 hours. Intake and output were monitored, and fluids were replaced as needed after the measurement of urine output and emesis. Before and after the drug administration, BUN, serum electrolytes, and serum creatinine were determined.

Audiograms, creatinine clearances, and cbcs were repeated before each treatment. Fifteen patients with locally invasive bladder tumors were treated with intravesical instillation of 100 mg of *cis*-platinum mixed with 500 ml of distilled water. This was given in a continuous irrigation lasting 4 hours every week for 6 weeks and every 2 weeks thereafter.

EVALUATION OF RESPONSES

Responses were defined as follows: *Complete clinical remission*—Disappearance of all measurable lesions with no clinical evidence of tumor remaining and the return of elevated serum markers to normal levels (testicular tumors). *Partial objective response*—Fifty percent decrease in the size of a lesion measured in 2 diameters with no increase in the size of any other lesions and/or appearance of new lesions. *Minimal objective response*—Twenty-five percent decrease in the size of a lesion with no increase in the size of any other lesion and/or appearance of new lesions. *Subjective response*—Decrease or disappearance of pain with no increase in the size of any existing lesion. *Stabilization*—No variation in the size of any lesion for a minimal period of 2 months (testes tumor) or 6 months (bladder and prostate carcinomas). *Progression*—Increase of at least 25% in the size of a measurable lesion and/or deterioration of the general condition.

Variation of the enzyme levels (acid and alkaline phosphatase and LDH) and/or biologic markers (carcinoembryonic antigen) was not considered reliable enough to be indicative of the evolution of some diseases (carcinoma of the prostate).

TABLE 1.—Response of patients with advanced testicular tumor treated with *cis*-platinum alone

Response	No. of— Patients	Recurrences	Tumor deaths	Patients alive with no evidence of disease	Survival time (mos)
Complete	6	6	6	0	4–8; median, 5.5
Partial	4	4	4	0	Median, 2
None	0	0	0	0	
Totals	10	10	10		

RESPONSE

Nonseminomatous Testicular Tumors

Treatment with cis-platinum alone (ten patients).—An objective response was observed in all ten patients (100%). Of these, six patients (60%) achieved a complete clinical remission which lasted from 4 to 8 months, with an average of 5.5 months (table 1).

Treatment with cis-platinum, vincristine, bleomycin, prednisone, and actinomycin D (66 patients).—A complete clinical remission was seen initially in 54 patients (81.8%). Forty-one patients (62.1%) have remained in complete clinical remission for 6–40 months (13 patients have been disease-free for > 2 years and 20 patients have been disease-free for > 1 year). Thirteen patients had recurrence and died from their disease. In the 12 remaining patients a partial objective response was seen which lasted for an average of 2–30 months. All but one of these patients died from the uncontrollable progression of their disease (table 2).

Seminomas

In four patients (80%) a complete clinical remission was seen which lasted from 2 to 20 months (average, 10). One patient had a partial response which lasted 7 months; he then had disease recurrence and died.

Prostate Carcinoma

Estrogen-resistant group.—A partial objective response was observed in 17 of 54 patients (31.4%). This response lasted from 2 to 18 months with an average of 7 months. In addition, 20 patients (37%) had a subjective

TABLE 2.—Response of patients with advanced testicular tumor treated with *cis*-platinum, bleomycin, vincristine, prednisone, and actinomycin D

Response*	Treatment†	No. of— Recurrences	Tumor deaths	Patients alive with no evidence of disease	Survival time
CR (81.8%)	Chemotherapy only, 16 patients (24%) Chemotherapy and surgery, 37 patients (56%) Chemotherapy, surgery, and RT, 1 patient (1.5%)	13 (19.6%)	13 (19.6%)	41 (62.12%)	6–40 mos; 13 patients > 2 yrs; 20 patients > 1 yr; median, 19.5 mos
PR (18.2%)	Chemotherapy only, 0 patients Chemotherapy and surgery, 12 patients Chemotherapy, surgery, and RT, 0 patients	12 (18.2%)	12 (18.2%)	0	2–30 mos; median, 7.5 mos
NR	0 patients	—	—	—	
Totals	66 patients	25	25	41	

*CR = complete objective response; PR = partial objective response; NR = no response.
†RT = radiation therapy.

TABLE 3.—Response of patients with stage D estrogen-resistant carcinoma of the prostate treated with *cis*-platinum alone

Response	No. of patients	Duration of response
Complete clinical	0	2–18 mos
Partial objective	17 (31.4%)	2–18 mos; median, 7 mos
Subjective (decrease or disappearance of bone pain)	20 (37%)	1–17 mos; median, 6 mos
Stabilization	7 (12.9%)	6–9 mos; median, 7 mos
None	10	
Total	54	

response (significant decrease or disappearance of bone pain and return to ambulation without narcotics for pain control) which lasted from 1 to 17 months. Seven patients (13%) remained stable for 6–9 months, and ten patients did not respond to treatment (table 3).

Previously untreated group.—Thirteen of 32 patients (40.6%) treated with the combination of *cis*-platinum, diethylstilbestrol (1 mg/day), and orchiectomy showed a partial objective response lasting from 6 to 28 months. Twelve patients (37.5%) had a subjective response shown by the decrease or disappearance of bone pain for 6–26 months (average, 11), and seven patients remained stable (table 4).

Bladder Carcinoma

One complete clinical remission lasted 5 months (the patient then died of myocardial infarction); 18 partial remissions were also seen in this group. The total response was 19 of 51 patients (37.2%). The duration of the responses varied from 2 to 13 months, with an average of 5 months. Disappearance of pain (subjective remission) was seen in two patients (3.8%). Ten patients remained stable for 6–8 months (average, 6.5). Twenty patients did not respond to treatment (table 5).

TABLE 4.—Response of patients with stage D carcinoma of the prostate (previously untreated) to combination therapy with orchiectomy, estrogen therapy, and *cis*-platinum

Response	No. of patients	Duration of response
Complete	0	
Partial subjective	13 (40.6%)	6–28 mos; median, 11 mos
Subjective	12 (37.5%)	6–26 mos; median, 10 mos
Stabilization	7 (21.8%)	Median, 8 mos
None	0	
Total	32	

TABLE 5.—Response of patients with advanced bladder carcinoma treated with *cis*-platinum

Response	No. of patients	Duration of response
Complete clinical	1 (1.9%)	5 mos (patient died of myocardial infarction)
Partial objective	18 (35.2%)	2–13 mos; average, 5 mos
Subjective (disappearance of pain)	2 (3.9%)	Average, 2 mos
Stabilization	10 (19.6%)	6–8 mos; average, 6.5 mos
None	20 (39.2%)	
Total	51	

Renal Cell Carcinoma

None of the patients in this group responded to treatment. All died from the uncontrollable progression of their disease.

Miscellaneous Tumors

Rhabdomyosarcoma.—One patient showed a partial objective remission and remained stable with residual disease for 30 months. The two other patients did not respond to the treatment.

Penile carcinoma.—A partial objective response was seen in two of three patients with carcinoma of the penis. This response was short and lasted 2 months.

Adrenal carcinoma.—The patient with adrenal carcinoma showed a 50% reduction in the size of a single nodule in the lung and has remained in remission for 6 months.

Intravesical instillation of cis-platinum in the bladder.—A partial objective response was seen in eight patients (53.3%). This response lasted from 2 to 30 months. Most of the patients relapsed within a period of 3 months. The remaining seven patients in this group did not show any response to the treatment.

TOXICITY

All of the patients had varying degrees of nausea and vomiting. The intensity of these symptoms was not constant, and some of the patients received *cis*-platinum without vomiting at all. Mild to moderate renal toxicity shown by an increase in serum creatinine and BUN and by a decrease in creatinine clearance was observed in ten patients (4%).

TABLE 6.—Toxicity in the patients treated with *cis*-platinum alone and in combination

Toxic effect	No. of patients treated with—		
	cis-Platinum only (%)	*cis*-Platinum, vincristine, bleomycin, prednisone, and actinomycin D (%)	Totals (%)
Nausea	184 (100)	66 (100)	250 (100)
Vomiting	184 (100)	66 (100)	250 (100)
Renal toxicity	8 (4.3)	2 (3)	10 (4)
Hearing loss	40 (21.7)	2 (3)	42 (16.8)
Increased uric acid	50 (27)	2 (3)	52 (20.8)
Leukopenia	31 (16.8)	19 (28.7)	50 (20)
Thrombocytopenia	18 (9.7)	0	18 (7.2)
Anemia	50 (27)	6 (9)	56 (22.4)
Peripheral neuropathy	2 (1)	0	2 (0.8)
Alopecia	0	66 (100)	66 (26.4)
Mucositis	0	15 (22.7)	15 (6)
Lung fibrosis	0	0	0
Seizures	0	1 (1.5)	1 (0.4)
Allergy	0	10 (15.1)	10 (4)

Hearing loss in the high-frequency range was seen in 42 patients (16.8%). Five patients (2%) had to interrupt treatment because of hearing loss within the speech-frequency range. Uric acid elevation was seen in 52 patients (20.8%). They responded well to treatment with allopurinol and sodium bicarbonate. This was probably an expression of tissue destruction rather than direct *cis*-platinum toxicity. Mild to moderate leukopenia was seen in 50 patients (20%). Two patients had severe leukopenia (400 cells/mm^3). Eighteen patients (7.2%) had thrombocytopenia (< 100,000 cells/mm^3). One patient required platelet transfusion (< 30,000 platelets/mm^3). Fifty-six patients (22.4%) developed normocytic, normochromic anemia after several months of *cis*-platinum treatment. They were treated with blood transfusions and remained stable when *cis*-platinum therapy was discontinued. Forty-two of the patients with myelosuppression had previously been treated with radiation and/or myelosuppressive drugs before starting *cis*-platinum therapy. Two patients developed peripheral neuropathy, and one patient had grand mal seizures after *cis*-platinum administration (no lesions were present in the brain). Ten patients developed allergic reactions during the *cis*-platinum administration. They were treated with infusions of the steroid, Solu-Medrol (100–500 mg), and with an antiallergic, Benadryl. *cis*-Platinum was not discontinued in these patients, but they received antiallergic treatment (steroids and Benadryl) for 5 days before each *cis*-platinum treatment. Alopecia was observed in all 66 patients who received bleomycin. Fifteen patients developed mucositis. None developed lung fibrosis. The detailed toxic effects for patients receiving *cis*-platinum therapy alone and in combination are shown in table 6.

Our method of *cis*-platinum administration has proven to be effective in decreasing renal toxicity, nausea, and vomiting (2). This is probably due to the low level of circulating *cis*-platinum which remains below the toxic threshold. In addition, the use of mannitol reverses the osmotic gradient in the renal tubules (3) blocking the absorption of water, sodium, and *cis*-platinum contained in the glomerular filtrate. This method has been reliable and practical enough to be used in the outpatient department. Reports of instability of *cis*-platinum in < ½ normal saline solution (4) have prompted us to increase the sodium content of our solutions. We now use 5% dextrose in ½ normal saline.

Because of prolonged myelotoxic effects, we have modified our initial drug combination for testicular tumors and replaced vinblastine with vincristine. Since then, myelotoxicity has decreased; the therapeutic effects do not seem to be different from those reported by other investigators using *cis*-platinum in combination with vinblastine (5).

The fact that 56 patients (22.4%) developed anemia during the course of *cis*-platinum treatment seems to indicate that *cis*-platinum may have depressive effects on erythropoiesis. This effect could be similar to the anemia produced by chronic heavy metal intoxication.

Patients receiving *cis*-platinum intravesically showed no evidence of local or systemic toxicity.

CONCLUSIONS

The number of rhabdomyosarcoma and penile carcinoma patients treated in our series is limited and does not permit a full appreciation of the value of *cis*-platinum in these diseases. Our preliminary results may warrant further investigation using a larger number of patients.

Our response in the adrenal carcinoma patient is anecdotal, but such an observation may have some importance for future studies in view of the unresponsiveness of this disease to other drugs.

cis-Platinum has been disappointingly ineffective for the treatment of renal cell carcinoma. This is surprising because of its high concentration in the kidneys and its toxic effects on the normal renal tubular cells, which are the cells that become malignant when renal cell carcinoma develops.

In conclusion, *cis*-platinum appears to be effective for the treatment of nonseminomatous testicular tumors, seminoma, prostate and bladder carcinomas, and squamous cell carcinoma of the penis. It may be of value in rhabdomyosarcoma and adrenal carcinoma.

cis-Platinum is not effective for the treatment of renal cell carcinoma. Combination with other drugs may further enhance its efficacy.

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cis-Dichlorodiammineplatinum(II) in Non-Small Cell Carcinoma of the Lung [1]

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SUMMARY

The role of *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) as a single agent in non-small cell lung cancer has not been clearly defined, and extensive phase II studies have not been conducted. However, objective responses were noted in several early clinical trials. A review of these reports, including combinations with conventional agents, shows 15 partial responses among 100 patients. Results are presented on a new protocol using high-dose *cis*-platinum with mannitol-induced diuresis combined with cyclophosphamide and Adriamycin. An overall partial response rate of 28% was observed, including a 38% response rate in good performance status patients, with a median survival of 16 months in responding patients. Data from recent combination protocols do not define the activity of *cis*-platinum as a single agent, but suggest that it may be useful in non-small cell carcinoma of the lung.

[Cancer Treat Rep 63:1585–1588, 1979]

Because bronchogenic carcinoma remains a major problem in cancer treatment, development of new drugs has become increasingly important. Interest in clinical studies with *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) was generated by the antitumor activity seen in several experimental animal systems, including Lewis lung tumor, B16 melanoma, Walker 256 carcinosarcoma, sarcoma 180, and dimethylbenzanthracene-induced mammary carcinoma (1,2).

Although extensive phase II trials using *cis*-platinum in non-small cell lung cancer have not been completed, several investigators noted partial remissions during initial clinical studies (3–13). With reports in murine tumor models of synergism of *cis*-platinum with cyclophosphamide (14) and with Adriamycin (11), several two- and three-drug clinical studies were undertaken. Table 1 shows the responses noted in phase I–II trials in non-small cell lung cancer. Where histologic types were not mentioned or were not consistent with the World Health Organization classification, responses and numbers of patients entered are included under a "not specified" category. Responses are listed according to treatment regimen: *cis*-platinum alone (6,8,9,15–17), or with Adriamycin (11,12), cyclophosphamide (7), 5-fluorouracil (5), or vinblastine plus bleomycin (10). In all categories, *cis*-platinum doses varied from 10 mg/m^2 daily for 5 days to 120 mg/m^2 as a single dose. Courses were repeated every 3–4 weeks. The majority of patients were previously treated with chemotherapy and had advanced disease. There was a 21% (11 responses among 53 patients) response rate in those patients in whom histologic types were known; a 15% (15 responses among 100 patients) response rate was noted in all patients.

Recently, combination regimens with *cis*-platinum have been reported (18,19). These studies have included untreated patients with a good performance status (PS); major response rates of 18%–38% were observed. Our study of high-dose *cis*-platinum with mannitol-induced diuresis, combined with Adriamycin and cyclophosphamide (CAP), is reported below.

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TABLE 1.—Responses in phase I–II trials of *cis*-platinum in non-small cell lung cancer

Histologic type	No. of patients responding after treatment with—* *cis*-Platinum alone	*cis*-Platinum + 1 agent†	*cis*-Platinum + 2 agents†	Total No. of responses*	Response rate
Adenocarcinoma	3 (23)	1 (7)	1 (4)	5 (34)	15%
Epidermoid carcinoma	1 (3)	2 (4)	0 (1)	3 (8)	38%
Large cell undifferentiated	0 (6)	3 (5)	—	3 (11)	27%
Not specified	3 (43)	1 (4)	—	4 (47)	9%
Totals	7 (75)	7 (20)	1 (5)	15 (100)	15%

*Nos. in parentheses = total No. of patients treated.
†Conventional drugs (*see* text).

TABLE 2.—CAP regimen in non-small cell lung cancer: patient characteristics (46 patients)

Age in yrs	
Range	32–71
Median	50
Sex	
Males	29
Females	17
PS	
40–50	12
60–70	21
80–90	13
Prior therapy	
Chemotherapy only	4
Radiation therapy only	16
Radiation therapy and chemotherapy	8
None	18
Histologic type	
Adenocarcinoma	30
Epidermoid carcinoma	13
Large cell undifferentiated carcinoma	3

MATERIALS AND METHODS

Fifty patients with advanced non-small cell carcinoma of the lung were entered in this trial from March 1977 to March 1978. Eligibility required a microscopic diagnosis of carcinoma of the lung, objectively measurable disease, creatinine clearance of ≥ 70 ml/minute, PS ≥ 40 (Karnofsky scale), and acceptable hematologic status (wbc count ≥ 4000/mm^3 and platelet count ≥ 120,000/mm^3). Characteristics of the 46 adequately treated patients are shown in table 2.

All drugs were administered iv on Day 1, with cyclophosphamide at 600 mg/m^2, Adriamycin at 40 mg/m^2, and *cis*-platinum at 120 mg/m^2 with mannitol-induced diuresis by the method of Hayes et al (13). Initially, chemotherapy was repeated in 4 weeks and thereafter every 6 weeks.

Response criteria were defined as follows: *complete remission*—disappearance of all evidence of disease for ≥ 1 month; *partial remission (PR)*—> 50% decrease of all measurable disease for ≥ 1 month; *minor response*—objective (> 25%) tumor decrease, but less than that required for a PR; and *progression*—objective increase in measurable disease or the appearance of new tumor lesions.

RESULTS

Thirteen of the 46 adequately treated patients experienced PRs, for an overall response rate of 28% (table 3). No complete remissions occurred, but eight patients had minor objective responses. The PR rate was highest among patients with epidermoid carcinoma (38%), with no major responses observed in the three patients with large cell undifferentiated carcinoma.

Prior treatment with chemotherapy had a negative influence on PR rate, with only one of 12 (8.5%) patients responding; 12 of 34 (35%) patients who had not previously received chemotherapy had PRs. In addition, none of the 12 patients with a PS of < 60 had a PR. Thus, a 38% PR rate was observed in 34 patients with a PS of 60–90.

Duration of PRs ranged from 1 to 14 months, with a median of 8 months. Median survival from start of protocol was 6 months for all patients. Nonresponding patients survived a median of 4 months, while the median survival for those with PRs was 16 months.

TOXICITY

Toxicity was prominent with this protocol and the major manifestations observed are shown in table 4. Leukopenia occurred frequently, with a median wbc nadir of 2200/mm^3 (range, 200–10,200/mm^3) on Day 14. The median platelet nadir was 70,000/mm^3 (range, 5000–261,000/

TABLE 3.—Responses to CAP by histologic type in non-small cell lung cancer

Histologic type	No. of patients	No. of responses				Partial response rate
		Complete	Partial	Minor	Progression	
Adenocarcinoma	30	0	8	5	17	27%
Epidermoid carcinoma	13	0	5	2	6	38%
Large cell undifferentiated carcinoma	3	0	0	1	2	0
Totals	46	0	13	8	25	28%

TABLE 4.—CAP regimen in non-small cell lung cancer: toxicity in 46 patients

	No. of patients
Wbc nadir (cells/mm^3)	
< 1000	13
1000–1999	8
2000–2999	9
≥ 3000	16
Platelet nadir (cells/mm^3)	
≤ 30,000	12
31,000–50,000	5
51,000–75,000	10
≥ 76,000	19
Azotemia, maximum serum creatinine (mg/100 ml)	
2.0–2.9	4
> 3.0	3
Hearing loss	2

mm^3) on Day 13. Anemia was generally not a clinical problem, with a median fall in hemoglobin of 1.5 g/100 ml. Recovery of low blood counts occurred rapidly in most instances. However, three previously treated patients with low initial PSs died with septicemia while leukopenic within the first 2 weeks of starting the CAP protocol. These three patients were unevaluable for therapeutic effect, as was a fourth patient who was lost to followup.

Most patients experienced transient elevation of serum creatinine, < 50% increase above baseline values, with 15% of patients having a maximal serum creatinine of ≥ 2.0 mg/100 ml. One patient had marked drug-related renal toxicity which led to death. Clinically significant ototoxicity occurred in only two patients although evidence of drug-related effects was seen in the audiograms of 20% of patients.

All patients had nausea and vomiting which were moderately severe and were occasionally prolonged. There were no instances of hemorrhagic cystitis or cardiotoxicity.

DISCUSSION

Adriamycin and cyclophosphamide have been combined with *cis*-platinum in two prior studies (18,19); table 5 compares the results of these trials with those of the present study. Both prior protocols differed primarily by using lower doses of *cis*-platinum (40 or 50 mg/m^2); Bjornsson et al (19) included vincristine, as well as immunotherapy, in two thirds of the patients. Eagan et al (18) noted that previously untreated patients had a greater likelihood of response. Their high response rate of 38% may be due to the enlistment of the highest percentage of untreated patients of any of the three protocols. In addition, our study indicates that previously treated patients with a poor PS had few responses and were more likely to have severe toxicity. Although response rates of 28% and 38% in two of the three studies are favorable, the median survivals for responding patients of 16 months in our trial and > 1 year in Eagan's study (5–6 months for the whole group) are the most encouraging statistics. With the results summarized in

TABLE 5.—Comparison of protocols combining *cis*-platinum with Adriamycin and cyclophosphamide (CAP regimen)

	Eagan et al (18): CAP	Bjornsson et al (19): CAP + vincristine	Present study: CAP*
No. of evaluable patients	69	61	46
Response rate	38%	18%	28%
Median survival (mos)			
Whole group	5	5	6
Responding patients	12+	12	16

*High-dose *cis*-platinum + mannitol diuresis.

table 5, there is no evidence of an improvement in response rate with the high-dose *cis*-platinum regimen in non-small cell lung cancer.

Although many early studies with *cis*-platinum in lung cancer reported objective responses, these did not occur with the same frequency as did those observed in testicular, ovarian, or head and neck carcinomas (20,21). This evidence of tumor regression motivated investigators to study *cis*-platinum in combination with conventional agents. Data from recent combination protocols suggest that *cis*-platinum may be a useful agent in non-small cell carcinoma of the lung; however, only studies in patients with a good PS who have not received prior chemotherapy will establish the true response rate of *cis*-platinum as a single agent.

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Phase II Study of Cyclophosphamide, Adriamycin, and *cis*-Dichlorodiammineplatinum(II) by Infusion in Patients With Adenocarcinoma and Large Cell Carcinoma of the Lung [1]

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In 1975, we began an evaluation of the combination of cyclophosphamide, Adriamycin, and *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) (CAP-I) in patients with lung cancer. Both cyclophosphamide and Adriamycin had reported activity in these tumors (1). In addition, the combinations of cyclophosphamide plus Adriamycin (2), cyclophosphamide plus *cis*-platinum (3), and Adriamycin plus *cis*-platinum (4) have shown evidence of therapeutic potentiation. In patients with no prior chemotherapy exposure, tumor regressions were documented in 39% of patients (16 of 41) with non-small cell lung cancer (5) and in 75% of patients (six of eight) with small cell lung cancer (6). We subsequently re-evaluated CAP-I in patients with advanced adenocarcinoma of the lung and obtained a 42% (eight of 19 patients) regression rate (7). Based on the encouraging regression rates noted in non-small cell lung cancer, we attempted to improve the CAP-I regimen by (*a*) increasing its antitumor activity by the addition of VP-16-213 (8) or by increasing the dose of *cis*-platinum by 50% to 60 mg/m^2 (while keeping the doses of cyclophosphamide and Adriamycin the same as those used in the CAP-I regimen, ie, 400 mg/m^2 and 40 mg/m^2 iv respectively), and (*b*) by decreasing its renal and gastrointestinal toxicity by the administration of *cis*-platinum (at the higher dose) as a 6-hour infusion with mannitol and iv fluids. The evaluation of cyclophosphamide, Adriamycin, and *cis*-platinum given by 6-hour infusion with mannitol (CAP-II) is the subject of this report.

MATERIALS AND METHODS

Forty-two patients, 22 with adenocarcinoma (ACA) and 20 with large cell carcinoma (LCC) of the lung, were entered in the study after a written consent was obtained. The patients' pretreatment characteristics are shown in table 1. The median age of all patients was 60 years. There were 20 males and 22 females. The Eastern Cooperative Oncology Group performance score (ECOG PS) was 0–1 in 67% of the patients and 2–3 in 33%. Patients with active cardiac disease, an ECOG PS of 4, and serum creatinine levels of $\geq$ 1.3 mg/dl were ineligible for this study. Twenty-two (52%) patients had measurable indicator disease and 20 had evaluable but nonmeasurable indicator disease. No patient had had prior chemotherapy.

The criteria of response were different for measurable and evaluable but nonmeasurable disease and have been defined in detail elsewhere (5). Measurable disease required that the tumor have clearly measurable perpendicular diameters. In these patients, the standard definitions of tumor response were used. Evaluable disease was defined as visible tumor which was not clearly measurable. The response categories for evaluable disease included regression (a definite decrease in tumor size), stability (no definite increase or decrease in tumor size and no new lesions), and progression (a definite increase in tumor size or the appearance of new disease). Evaluable disease observations required agreement by two observers.

The doses of drugs were 400 mg/m^2 (cyclophosphamide), 40 mg/m^2 (Adriamycin), and 60 mg/m^2 (*cis*-platinum). The cyclophosphamide was given by rapid iv infusion and was followed by 250 ml of 5% dextrose in water over the next 30 minutes. Three hours later, Adriamycin was given by rapid iv infusion and was immediately followed by *cis*-platinum given as two consecutive 3-hour infusions containing a total of 60 mg/m^2 of *cis*-platinum, 25 g of mannitol, and 1500 ml of 5% dextrose in ½ normal saline. Chemotherapy was repeated at 4-week intervals. The doses of cyclophosphamide and Adriamycin were adjusted for myelosuppression and the dose of *cis*-platinum was adjusted based on the value of the serum creatinine. The dose of *cis*-platinum used in patients with a serum creatinine level of $\leq$ 1.2 mg/dl was 60 mg/m^2. The dose of *cis*-platinum used in patients with a serum creatinine level of 1.3 or 1.4 mg/dl was 45 mg/m^2. For serum creatinine levels of 1.5–1.9 mg/dl, the dose of *cis*-platinum was 30 mg/m^2. *cis*-Platinum was permanently discontinued if a creatinine level of $\geq$ 2.0 mg/dl was observed. Adriamycin was permanently discontinued at the first sign of cardiac toxicity. Prochlorperazine was

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TABLE 1.—Pretreatment patient characteristics (42 patients)

	Cell type	
	ACA	LCC
No. of patients	22	20
Median age in yrs (range)	58 (38–69)	63 (41–70)
Sex (males:females)	10:12	10:10
ECOG PS (0–1:2–3)	15:7	13:7
Indicator disease (measurable:evaluable)	10:12	12:8

routinely administered (unless allergy existed) using the following schedule: 5 mg given im with cyclophosphamide and 10 mg given im at the start of *cis*-platinum. Four hours after the second im injection, prochlorperazine (either 25-mg suppositories or 15-mg Compazine) was administered every 8–12 hours until nausea subsided. During the first course of chemotherapy only, radiation therapy could be given in standard palliative doses to non-indicator lesions as clinically indicated.

RESULTS

Tumor Regressions

All 42 patients were evaluable for tumor response. The overall regression rate was 48% (20 of 42 patients). There was no difference between ACA and LCC, with 45% (ten of 22 patients) and 50% (ten of 20 patients) regression rates noted respectively. There were no complete regressions. The partial regression rates were 50% (11 of 22 patients) among those with measurable disease and 45% (nine of 20 patients) among those with evaluable disease.

Time to Disease Progression

Time to disease progression (from Day 1 of treatment) was estimated from Kaplan-Meier curves. The median time to disease progression for all 42 patients was 94 days (range, 11–336+) and was not significantly different for the two cell types. For all 42 patients, median time to disease progression among patients with tumor regression was 196 days (range, 29+ to 336+) versus 34 days (range, 11–301) for nonresponders.

Survival

Survival durations were estimated from Kaplan-Meier curves and analyzed using Gehan-Wilcoxon two-sided test statistics. The overall median survival for the 42 patients was 173 days (range, 11–456+). Responders with both cell types survived significantly longer ($P < 0.01$) than did nonresponders (> 340 versus 127 days). There were no significant differences between the two cell types with regard to any of the survival parameters.

TOXICITY

Nausea and vomiting of a moderate to severe degree were noted in all patients. In some patients, they continued for several days following the treatment.

Leukopenia occurred in 67% of patients after the first course of treatment. The median wbc nadir was 2600/mm^3 (range, 500–3900/mm^3). Thrombocytopenia occurred less frequently (in 20% of patients) with a median platelet nadir in those patients of 90,000/mm^3 (range, 53,000–119,000/mm^3). Anemia was also associated with the treatment. The median hemoglobin level for all patients at the start of treatment was 13.25 g/dl and fell following the first three treatments to median values of 12.15, 11.42, and 11.14 g/dl respectively. The biggest drop seemed to occur following the first two treatments.

Renal toxicity, as evidenced by a rise in serum creatinine to > 1.2 mg/dl, occurred in 19% of patients (eight of 42). The highest posttreatment creatinine level noted was 1.7 mg/dl. Among the eight patients who experienced an elevation, the serum creatinine returned to normal in two, was decreased in one, and either remained stable or was the last observation in five. *cis*-Platinum dose was decreased in patients experiencing increases in creatinine.

No unequivocal *cis*-platinum neurotoxicity was noted, but it was suspected in one patient. No tinnitus or clinically apparent hearing loss was observed. No Adriamycin-induced cardiac toxicity was noted.

DISCUSSION

The regression rate noted for the CAP-I regimen (5,7) in patients with previously untreated ACA and LCC was 38% (16 of 42 patients). The median survival for the 33 patients with ACA in that group was 218 days (range, 34–842+). The addition of VP-16-213 to the CAP-I regimen (8) in patients with ACA did not seem to increase either the regression rate (35%) or the overall median survival (191 days). In this current study, the overall regression rate of 48% is somewhat higher than that of previous studies, but the overall median survival is somewhat less (173 days). There were no obvious differences among the patients with regard to age, sex, PS, prior treatment, and type of indicator disease.

The regression rates noted for the various CAP regimens in patients with ACA and LCC were higher than the 0 regression rate using the combination of Adriamycin and *cis*-platinum reported by Mills et al (9), but were about the same as the 40% partial regression rate reported by Vogl et al (4). Issell et al (10) recently reported on the combination of ftorafur, Adriamycin, and

cis-platinum. They noted a 42% (ten of 24 patients) complete and partial regression rate in previously untreated patients with ACA and LCC lung cancer, with a projected overall median survival of about 25 weeks. These data seem quite comparable to the CAP data.

Increasing the dose of *cis*-platinum in the CAP-II regimen has not obviously improved the CAP regimen in any major way. Gralla et al (11) reported on a variant of the CAP regimen in which the *cis*-platinum dose was twice that used in the CAP-II regimen. They noted a 30% regression rate in patients with ACA, but some had had prior chemotherapy. We have previously reported only a 9% (two of 22 patients) regression rate in ACA with *cis*-platinum alone (7). At the present time, we feel that the role of *cis*-platinum in the CAP regimen is not completely settled. We believe that it is important for the full activity of the combination, but we are uncertain about the best dose level and method of administration. In a future protocol, we will explore two different dose levels. In addition, we feel that the gastrointestinal and renal toxic effects of the 40-mg/m^2 rapid iv infusion schedule and the 60-mg/m^2 by 6-hour infusion schedule of *cis*-platinum are quite similar, and that neither schedule can be conveniently used for prolonged therapy (> 6 months) because of the gastrointestinal toxicity. Some of our early (unpublished) data suggest that a 3-day schedule using 1-hour infusions may be best from an overall toxicity standpoint.

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cis-Dichlorodiammineplatinum(II) and VP-16-213: An Active Induction Regimen for Small Cell Carcinoma of the Lung [1]

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SUMMARY

Thirty-eight patients with small cell carcinoma and no prior therapy were treated with a combination chemotherapy program including 60 mg/m^2 of *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) iv on Days 1 and 22 and 120 mg/m^2 of VP-16-213 iv on Days 4, 6, 8, 25, 27, and 29. This was followed by 1000 mg/m^2 of cyclophosphamide, 40 mg/m^2 of Adriamycin, and 1.4 mg/m^2 of vincristine, all given iv on Days 42, 63, 84, and 105. The program was then recycled, with *cis*-platinum and VP-16-213 beginning on Day 126 and the regimen repeated as above. All patients received prophylactic whole-brain radiation (usually at a dose of 3000 rads in ten fractions) between Days 42 and 63. The projected duration of treatment is 18 months in the absence of relapse. In 21 patients with limited disease, the complete response rate was 52% (5.75+–15.25+ months) and the partial remission rate was 48% (2.25–10.5 months). The extensive-disease group showed a complete remission rate of 41% (4.75–11+ months) and a partial remission rate of 47% (3.75–11.5+ months). Response to therapy with *cis*-platinum and VP-16-213 was very rapid and invariably maximal by the end of the 6-week induction period. Survival for the limited-disease group appears encouraging but followup time is insufficient. Toxicity included nausea and vomiting, myelosuppression, alopecia, and renal insufficiency which was dose-limiting in two patients. The *cis*-platinum and VP-16-213 combination is clearly an active induction regimen in small cell carcinoma of the lung, but whether it will play a role in increasing long-term survival rates remains to be seen.

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During the past few years interest in small cell carcinoma of the lung has sharply increased. At least 21 abstracts relating to the treatment of this lung cancer subtype were published in the 1978 Proceedings of the American Association for Cancer Research and the American Society of Clinical Oncology. The reasons for such interest are clear: radiation therapy and a wide variety of drugs can produce impressive reduction of tumor bulk and dramatic relief of related symptoms. Since combinations of agents have generally produced higher response rates than single-agent treatments, most current experimental programs have favored the use of combinations, with or without radiation therapy, with the obvious intent of increasing the complete remission (CR) rate and the frequency of long-term survival. Although this latter goal remains largely unfulfilled (1,2), some early data suggest that further improvement in survival of the treated population beyond the usual 8–12-month median may be possible (3,4). In any case, further progress in the control of this tumor will very likely depend, at least in part, on the identification of new active agents.

Recently, two groups have documented a high order of activity for VP-16-213 in small cell carcinoma (5,6); indeed, this agent appears as effective as any previously tested single agent. Although the extent of activity of

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cis-dichlorodiammineplatinum(II) (*cis*-platinum) had not yet been defined in small cell carcinoma, we decided to study the combination of *cis*-platinum and VP-16-213. Of six patients who had failed to respond to previous combination chemotherapy, we observed one CR, four partial responses (PRs), and one minor response (MR), with a median response duration of 11 weeks (7). We therefore elected to evaluate this regimen as initial therapy in sequential combination with conventional agents.

MATERIALS AND METHODS

A total of 38 patients with small cell carcinoma of the lung were studied. All patients had their diagnosis documented histologically or cytologically by the Department of Pathology at Memorial Sloan-Kettering Cancer Center. Other criteria for eligibility included a history of no prior invasive cancer, no previous chemotherapy, normal renal function (serum creatinine < 1.2 mg/dl), and adequate bone marrow reserve, with a total leukocyte count of $\geq 4000/mm^3$ and a platelet count of $\geq 100{,}000/mm^3$. Details of the initial staging workup have been described previously (2,8). All patients had at least one focus of measurable disease.

The treatment schedule consisted of 60 mg/m^2 of *cis*-platinum iv after prehydration with 500 ml of 5% dextrose and normal saline on Days 1 and 22; a mannitol-induced diuresis was not utilized. A dose of 120 mg/m^2 of VP-16-213 was given iv on Days 4, 6, 8, 25, 27, and 29. The second phase of therapy consisted of 1000 mg/m^2 of cyclophosphamide, 40 mg/m^2 of Adriamycin, and 1.4 mg/m^2 of vincristine, all given iv on Days 42, 63, 84, and 105. The treatment schedule was recycled, with *cis*-platinum and VP-16-213 beginning on Day 126 and the entire schedule repeated as outlined above. A standard dose-modification scheme was employed in the presence of drug-induced toxicity (table 1). The projected duration of treatment was 18 months in the absence of relapse. All patients received prophylactic whole-brain radiation (9), usually at a dose of 3000 rads in ten fractions, between Days 42 and 63. Radiation therapy to the primary complex, however, was reserved for patients who had disease progression with chemotherapy and was not used to improve or consolidate responses achieved with chemotherapy.

TABLE 1.—Dose-modification schedule

Toxicity	Drugs	Clinical signs and/or laboratory parameters			% of planned dose given
		Wbc count (cells/mm^3)		*Platelet count (cells/mm^3)*	
Myelosuppression	*cis*-Platinum	≥ 4000	and	≥ 150,000	100
	VP-16-213	3000–3999	or	100,000–149,999	50
	Cyclophosphamide	2500–2999	or	75,000–99,999	25
	Adriamycin	< 2500	or	< 75,000	0
		Creatinine level during therapy			
Nephrotoxicity	*cis*-Platinum	< 1.2 mg/dl			100
		1.2–1.6 mg/dl			75
		1.7–2.0 mg/dl			50
		> 2.0 mg/dl			0
		Bilirubin level during therapy			
Hepatotoxicity	Adriamycin	< 2.0 mg/dl			100
	Vincristine	2.0–5.0 mg/dl			50
		> 5.0 mg/dl			0
		Neurologic symptoms			
Neurotoxicity	Vincristine	Absent deep tendon reflexes and mild-to-moderate paresthesias			100
		Severe paresthesias or mild muscle weakness			50
		Disabling muscle weakness, ileus, and urinary retention			0
		Cardiac symptoms			
Cardiotoxicity	Adriamycin	Signs of congestive heart failure and cardiac arrhythmias			Stopped
		Nonspecific ST- and T-wave abnormalities			Continued

Response categories are as previously defined (2) and were determined by monthly physical examinations, sequential chest roentgenograms, blood chemistries, and followup scintiscans. Cbcs, serum creatinine, liver function tests, and electrocardiograms were repeated periodically to monitor toxicity related to treatment. Duration of response was measured from the start of therapy to the time of initial evidence of relapse or progression.

RESULTS

From April 1977 to March 1978, 38 patients satisfied the eligibility criteria. This group consisted of 11 females and 27 males, ranging in age from 36 to 84 years (mean, 57; median, 58). Twenty-one patients had disease confined to the ipsilateral hemithorax, including the hilar, mediastinal, and/or supraclavicular lymph nodes, while 17 patients had extensive disease beyond these confines. Performance status (10) ranged from 60 to 100 (median, 90). With the exception of six patients who began therapy at 1.5, 1.5, 1.75, 2, 2.5, and 2.75 months after diagnosis, all patients began therapy within 1 month of diagnosis.

Responses are outlined in table 2. Of the 21 patients with limited disease, there were 11 CRs lasting from 5.75+ to 15.25+ months, and ten PRs lasting from 2.25 to 10.5 months. The median duration of PR is 4.5 months but that of CR has not yet been reached. Of the 17 patients with extensive disease, there were seven CRs lasting from 4.75 to 11+ months, eight PRs lasting from 3.75 to 11.5+ months, and two MRs lasting 1.5 and 6 months. In the extensive-disease group the median duration of CR was 5.25 months and the median duration of PR was 4 months. Thus, the major response rate (CR + PR) for the total patient population was 95%.

TABLE 2.—Response to chemotherapy

Type of response	No. of patients	Duration of response in mos*
CR		
Extensive disease	7	4.75, 4.75, 4.75+, 5.25, 7, 8, 11+
Limited disease	11	5.75+, 6+(2), 8+(2), 8.25+, 8.5+, 9, 11.75+, 13.75+, 15.25+
PR		
Extensive disease	8	3.75(2), 4(2), 5.5, 6.5, 8.25, 11.5+
Limited disease	10	2.25, 3, 3+, 4.5(2), 5, 7.5, 9.25+, 10, 10.5
MR		
Extensive disease	2	1.5, 6
Limited disease	0	
Total	38	

*Nos. in parentheses = No. of patients.

In all cases, serial chest roentgenograms demonstrated a rapid response to therapy with *cis*-platinum plus VP-16-213. Most patients showed substantial objective improvement radiologically within 1 week of the start of therapy. In all patients, chest roentgenograms showed that the maximal tumor response developed during the initial phase of treatment with the first two cycles of *cis*-platinum and VP-16-213; no further improvement of the extent of response could be documented with subsequent chemotherapy.

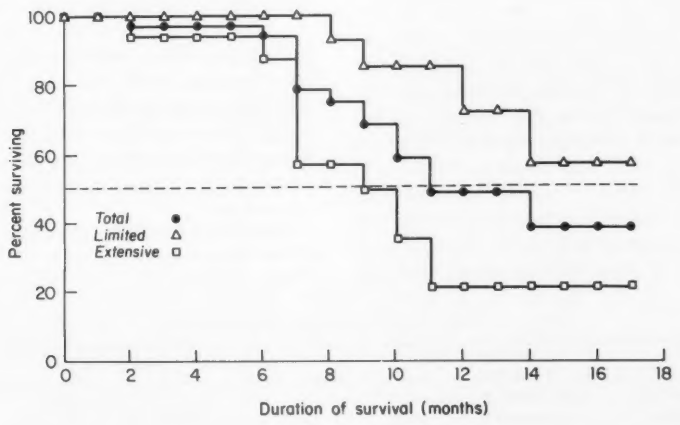

FIGURE 1.—Survival curves for the total patient population and for the limited- and extensive-disease subgroups. Survival times are measured from start of therapy. The adjusted No. of patients at risk for representative time points are: at start of treatment, 38 patients; at 6 mos, 30 patients; at 9 mos, 14.5 patients; and at 12 mos, 6 patients.

The survival curves for the total patient population and the limited- and extensive-disease subgroups are shown in figure 1. The median survival of the total population was 12 months from diagnosis and 11 months from the start of therapy. Patients presenting with extensive disease had a median survival from the start of therapy of 9 months; for those patients with limited disease, the median survival has not yet been reached. An analysis of sites of disease progression in those patients in whom this occurred is shown in table 3. As of August 1978, 23 patients had either died or developed progressive disease. In these 23 patients, 33 sites of initial relapse were detected, including lungs and/or mediastinum (21), liver (three), brain (three), peripheral lymph nodes (four), and bone (two). Those patients with limited disease tended to have recurrence predominantly at the site of the primary tumor complex without associated extrathoracic progression, while those patients presenting initially with extensive disease had a greater tendency to have progression simultaneously at both intrathoracic and extrathoracic sites.

The toxicity of this regimen included nausea and vomiting, which was universal and generally most severe with *cis*-platinum. Although most patients were distressed by this, only one found therapy intolerable and after 5 months in the protocol refused further therapy. Alopecia occurred in all patients. The extent of myelosuppression is shown in table 4. Since not all patients returned to the clinic for blood cell counts at the time of their expected nadirs, these figures are obviously minimum estimates. Leukopenia, and especially thrombocytopenia, occurred during therapy with *cis*-platinum plus VP-16-213. Only two of these patients developed episodes of sepsis requiring hospitalization and treatment with iv antibiotics. Both patients recovered from their infections only to die shortly thereafter of progressive cancer. Only one of the nine patients with platelet counts of $< 50{,}000/mm^3$ had significant gastrointestinal bleeding which required hospitalization for rbc and platelet transfusions. The average hemoglobin fall during chemotherapy was 4 g/dl (mean hemoglobin at the start of therapy, 13.5 g/dl). Two patients showed creatinine rises of > 2 mg/dl during therapy. One of these patients died of meningeal carcinomatosis after his serum creatinine had leveled off at 6–7 mg/dl. The other patient's maximum creatinine was 4.4 mg/dl but returned shortly to 2 mg/dl. Most patients experienced mild signs of vincristine neuropathy but only one required temporary cessation of this agent because of refractory constipation. Neither stomatitis nor any abnormalities of liver function attributable to drug therapy occurred.

TABLE 4.—Hematologic toxicity

Hematologic toxicity	No. of patients	
	cis-Platinum/ VP-16-213	CAV*
Wbc count (cells × $10^3/mm^3$)		
≥ 4	8	19
3–3.9	12	7
2–2.9	13	5
1–1.9	4	2
< 1	1	1
Platelet count (cells × $10^3/mm^3$)		
≥ 150	23	30
100–149	4	1
50–99	3	2
< 50	8	1

*Cyclophosphamide, Adriamycin, and vincristine.

DISCUSSION

The present program has produced both a higher total response rate and a greater proportion of CRs than any of our previous regimens for small cell carcinoma of the lung (2,11).[3] Although the median survival of the total population approximates that of previous studies, the survival of the limited-disease subgroup appears somewhat better than any results we have achieved to date. Firm conclusions concerning increased survival will, of course, have to await a suitable interval for prolonged followup.

Particularly encouraging has been the relative freedom from severe acute toxicity necessitating hospitalization, which has been a feature of other vigorous treatment plans for this disease (3,12). In addition, we seem to have had little problem with cumulative nephrotoxicity, perhaps because of the 12-week periods of rest after *cis*-platinum administration. Alternatively, the potential for cumulative renal injury may become more evident as followup time increases and more patients are exposed to higher total doses of *cis*-platinum.

[3]Sierocki JS, Hilaris BS, Hopfan S, et al. Small cell carcinoma of the lung—experience with a six-drug regimen. *Cancer*. In press.

TABLE 3.—Sites of disease progression

Extent of disease	No. of patients with disease progression	Sites of disease progression				
		Lung	Liver	Brain	Lymph nodes	Bone
Extensive	14	12	3	3	3	2
Limited	9	9	0	0	1	0

Since the activity of *cis*-platinum as a single agent has not yet been defined in small cell carcinoma, its therapeutic role in combination with VP-16-213 is speculative. Since the response rate of small cell carcinoma to VP-16-213 alone is 35%–50% (5,6,13), it seems probable that *cis*-platinum plus VP-16-213 is more active than VP-16-213 alone. Whether the combination is superior to *cis*-platinum alone, however, can only be answered by a suitable trial in which the regimens are compared in similar patient groups. Although other investigators employing different schedules of administration of VP-16-213 have noted thrombocytopenia (6,13), Eagan et al (5), utilizing the same VP-16-213 schedule as in the present study, noted no thrombocytopenia. The impressive frequency of thrombocytopenia in our experience with *cis*-platinum plus VP-16-213, which is certainly far greater than that expected from *cis*-platinum alone, may therefore suggest that this combination produces synergistic toxicity.

Analysis of relapse patterns reveals a marked tendency for patients with limited disease to relapse at the primary complex, where the initial involvement was gross. This finding, also noted previously,[3] suggests that the routine use of radiotherapy early, but not necessarily initially, in the treatment plan may decrease the rate of local relapse and perhaps result in an increased cure rate. That the combination of radiotherapy and chemotherapy will not be a simple matter, however, is already well-known, both because of a dose-effect relationship between radiation therapy and local control in small cell carcinoma (14) and because of the ample potential for vastly increased toxicity when the two treatment modalities are given simultaneously (15,16).

In conclusion, the combination of *cis*-platinum and VP-16-213 appears to be as active an induction regimen for small cell carcinoma of the lung as any combination of drugs currently in use. Speculation about its ultimate effect on long-term survival, however, is clearly premature.

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Phase II Trial of *cis*-Dichlorodiammineplatinum(II) in Advanced Malignant Lymphoma and Small Cell Lung Cancer: Preliminary Results

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SUMMARY

In an ongoing cooperative study of the Cancer and Acute Leukemia Group B, 21 evaluable patients with advanced malignant lymphomas were treated with 70 mg/m^2 of *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) once every 3 weeks. All patients had received extensive prior therapy. Partial remission was obtained in two of seven patients with Hodgkin's disease, for 1+ and 7 months, and in three of 14 patients with non-Hodgkin's lymphoma, for 2, 2+, and 2.5 months. In another ongoing trial, 11 patients with advanced, pretreated small cell cancer of the lung received 80 mg/m^2 of *cis*-platinum once every 3 weeks. Four patients achieved partial remissions. These lasted 2+ and 2.5 months in the two patients evaluable for duration of response. Two further clear-cut tumor regressions were noted. The major toxic effects were myelosuppression and vomiting. In the second trial, one case of probable drug-related fatal nephrotoxicity was encountered despite optimal forced diuresis with mannitol and furosemide. *cis*-Platinum definitely warrants further evaluation in these diseases because of significant effectiveness even after extensive prior treatment.

[Cancer Treat Rep 63:1599–1603, 1979]

Despite the large amount of clinical work carried out in the last 5 years with this investigational drug, data on the effect of *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) in the treatment of small cell lung cancer and malignant lymphoma are scanty (1). Some efficacy for *cis*-platinum has been reported in lymphomas (2–6), but results of a systematic, disease-oriented phase II trial are still lacking. In the treatment of previously untreated patients with small cell cancer of the lung a very provocative response rate of 90% among 20 patients has been reported using the combination of *cis*-platinum and VP-16-213 (7). In another unpublished phase II study, only three minor responses among 22 previously treated patients with small cell cancer of the lung were noted. Only about one half of the patients received at least 60 mg/m^2 of the drug.[6]

These facts prompted us to propose a phase II clinical trial for advanced lymphomas to the Cancer and Acute Leukemia Group B and to carry out a study in small cell cancer of the lung at our institution.

PHASE II TRIAL IN ADVANCED LYMPHOMA

Methods and Materials

Patients with advanced histologically proven malignant lymphoma having measurable disease were eligible for the protocol. As of June 1978, 23 patients had been entered in this ongoing study. The pretreatment characteristics of the 23 evaluable patients are summarized in table 1. About 60% of the patients had received radiotherapy and three or more various types of combination

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[6]Hansen HH. Unpublished data.

TABLE 1.—*cis*-Platinum in the treatment of advanced lymphoma: pretreatment patient characteristics

Characteristics	No. of patients
No. of evaluable patients	21
Median age in yrs: 48 (range, 23–74)	
Sex	
Women	7
Men	14
ECOG* performance score	
0–1	9
2–3	12
Previous radiotherapy	12
Previous chemotherapy	
1 combination	4
2 combinations	4
3 combinations	10
4 combinations	3
Mean interval from diagnosis to start of therapy with *cis*-platinum in mos (range)	
Hodgkin's disease	72 (25–125)
Well-differentiated lymphocytic lymphoma	34 (20–48)
Poorly differentiated lymphocytic lymphoma	11 (10–12)
Diffuse histiocytic lymphoma	10 (6–16)
Mixed-cell type	36 (16,56)

*ECOG = Eastern Cooperative Oncology Group.

chemotherapy. Patients were considered evaluable at the time of the second scheduled course of *cis*-platinum.

Other criteria for eligibility were an absolute granulocyte count of $> 2000/mm^3$ and a platelet count of $> 100,000/mm^3$, except where low counts were clearly disease-related; a creatinine of < 1.5 mg/dl and a creatinine clearance of $> 75\%$ of the predicted value; and a total serum bilirubin of < 2 mg/dl and an SGOT of < 120 IU. Patients with well-differentiated lymphocytic lymphoma of the diffuse type had < 5000 lymphocytes/mm^3 in the peripheral blood and $< 30\%$ lymphocytes in the bone marrow.

The initial dose of *cis*-platinum was 70 mg/m^2 once every 3 weeks. The drug was injected through the tubing of a running infusion over a period of 20–60 minutes. Before treatment, urine output had to be at least 100 ml/hour for 4 consecutive hours without diuretics. Mannitol was given at a dose of 12.5 g iv immediately before *cis*-platinum and at a dose of 0–5 g/hour afterwards in order to maintain a urine output of at least 100 ml/hour for 8 hours from the start of the *cis*-platinum infusion.

Subsequent courses of *cis*-platinum were administered only if renal function values were or had returned to normal and if the nadir blood cell counts had been reached and the values were recovering. Drug dose modifications were based upon cbc results the day *cis*-platinum was to be administered. The dose was decreased by 50% if the wbc count was 3000–3999/mm^3 and/or the platelet count was 75,000–99,999/mm^3. The reduction was 75% if the wbc count was 2000–2999/mm^3 and/or the platelet count was 50,000–74,999/mm^3. Treatment was withheld if the wbc count was $< 2000/mm^3$ and/or the platelet count was $< 50,000/mm^3$. Five patients received one course, ten patients received two courses, three patients received three courses, and three patients received four or more courses (maximum, eight courses). The response to therapy was classified as complete remission (CR), partial remission (PR), stable disease, or increasing disease. Standard criteria were used, with a PR indicating a reduction in tumor size of $> 50\%$ (product of two perpendicular tumor diameters).

Results

Twenty-three patients from three institutions were entered in the lymphoma study. Two were considered inevaluable: one for protocol violation and one because of an inadequate trial (death occurred 1 week after the first dose). The therapeutic results are summarized in table 2. Two PRs were noted among seven evaluable patients with Hodgkin's disease. The durations of response were 1+ and 7 months. Among the 14 evaluable patients with non-Hodgkin's lymphoma (NHL) three PRs

TABLE 2.—Therapeutic results of *cis*-platinum in the treatment of advanced lymphoma (70 mg/m^2 q3wks with forced diuresis)

Diagnosis	No. of evaluable patients	No. of PRs	Response duration (wks)
Hodgkin's disease	7	2	4+, 29
Diffuse histiocytic lymphoma	6	2	8, 8+
Poorly differentiated lymphocytic lymphoma	3	—	—
Well-differentiated lymphocytic lymphoma	3	1	10
Mixed-cell type	2	—	—

were observed in the following subgroups: two of six with diffuse histiocytic lymphoma (durations, 2 and 2+ months), none of three with poorly differentiated lymphocytic lymphoma, one of three with well-differentiated lymphocytic lymphoma (duration, 2.5 months), and none of two with mixed NHL.

Myelosuppression and profuse vomiting were the major toxic effects. Five patients had a wbc count nadir $< 3000/mm^3$ and/or a platelet count nadir $< 75,000/mm^3$. In five other patients myelosuppression was only partially evaluable because of low pretreatment counts related to the disease. Prior to treatment the mean wbc count was $3200/mm^3$ and the mean platelet count was $52,000/mm^3$. In two of these patients the counts improved after treatment with *cis*-platinum was started. In the three other patients however, a further decrease of peripheral blood cell counts was registered, with a wbc count nadir $< 1000/mm^3$ (in two patients) and a platelet count $< 20,000/mm^3$ (in two patients).

The median nadir was seen on Day 14 (range, Days 7–28). The following course was postponed 1 week in three patients and 2 weeks in two other patients due to persisting low blood cell counts.

Nephrotoxicity proved to be manageable. Only one patient had permanent elevation of the creatinine to > 1.5 mg/dl. This occurred after the first course of therapy. Six patients showed a transient rise of the creatinine after *cis*-platinum administration. The values then returned to normal before the next course.

No hepatotoxicity or neurotoxicity was observed. Ototoxicity was not regularly monitored. One patient with stable disease refused further therapy after the first course because of persisting subjective malaise.

TABLE 3.—*cis*-Platinum in the treatment of small cell lung cancer: pretreatment patient characteristics

Characteristics	No. of patients
No. of evaluable patients	11
Median age in yrs: 59 (range, 50–71)	
Sex	
Women	2
Men	9
ECOG* performance score	
0–1	4
2–3	7
Previous chemotherapy	
1 combination	6
≥ 2 combinations	5
Stage of disease	
Limited	6
Extensive	5
Median interval from diagnosis to start of therapy with *cis*-platinum in mos (range)	9 (4–18)

*ECOG = Eastern Cooperative Oncology Group.

Discussion

Malignant lymphomas, even in their advanced stage, encompass such a variety of prognostic factors that phase II trials are always fraught with problems (8,9). The recent conflicting results with VP-16-213 in the treatment of these diseases once again underscored this fact (10).

In our current study we had two PRs among seven patients with Hodgkin's disease and three PRs among 14 patients with NHL. These data do not allow any definite conclusions to be made as to the degree of activity of *cis*-platinum in the treatment of malignant lymphomas. It is hoped that a rapid accrual of more patients will provide more information in the near future.

PHASE II TRIAL IN SMALL CELL LUNG CANCER

Methods and Materials

As of May 1, 1978, 11 patients with histologically proven small cell lung cancer had been entered in this study. The pretreatment patient characteristics are summarized in table 3. All patients were previously treated with at least one combination chemotherapy regimen. Disease staging was based upon physical examination, chest roentgenograms, blood chemistries, and bone marrow biopsy. Skeletal and liver scintigrams were performed when indicated. Patients with proven or suspected central nervous system disease were excluded. Criteria for eligibility and dose adjustment were identical to those of the above lymphoma study. The mode of *cis*-platinum administration was also somewhat similar, with the exception that furosemide (40–80 mg given over 6 hours) was added to the mannitol before starting the *cis*-platinum infusion. The starting dose of *cis*-platinum was 80 mg/m^2 once every 3 weeks. This ongoing study calls for treatment with *cis*-platinum for > 6 weeks unless earlier disease progression becomes life-threatening or complementary radiotherapy is judged to be mandatory.

Two patients received only one course of *cis*-platinum and then began treatment with complementary radiotherapy (*see* table 4). Five patients received two courses, three patients received three courses, and one patient received four courses of *cis*-platinum.

The same standard response criteria used in the lymphoma study were used in this study, with the sole exception that minor responses (MR) were also recorded.

MR is defined as a > 50% reduction in the product of two perpendicular diameters of a measurable lesion with a second indicator lesion remaining stable.

Results

All 11 patients were evaluable. The details of the tumor regressions are presented in table 4. We observed four PRs, all of them in patients with limited disease and a performance status of 1–2 (Eastern Cooperative Oncology Group scale). One of the patients, who achieved a PR with *cis*-platinum, had attained only an MR with prior combination chemotherapy consisting of cyclophosphamide, methotrexate, vincristine, and VP-16-213 (patient 3, table 4). The duration of remission was 5 months for the four-drug regimen and 2.5+ months for the *cis*-platinum regimen. In two patients with PR the duration cannot be evaluated because both patients received complementary radiotherapy after achieving PR. The first patient (No. 1, table 4) had two indicator lesions, one of which was a total atelectasis of the upper right lobe. This was converted to a residual atelectasis after one course of *cis*-platinum. The other lesion disappeared completely. In the second patient (No. 4, table 4) a superior vena cava syndrome disappeared after one course of *cis*-platinum and the measurable lesion decreased > 50% in size. In both cases complementary radiotherapy was thought to be indicated and *cis*-platinum will be resumed after its completion.

Two additional patients showed clear-cut MR which lasted 2 and 2+ months. In both cases huge liver metastases were hardly affected by the therapy, but the lung lesions shrank 50%. One of these two patients developed a hyperuricemia of 18.3 mg/dl and died of progressing renal failure 8 weeks after starting *cis*-platinum (patient 6, table 4). One patient had clearly progressive disease that stabilized for 2.5 months with *cis*-platinum treatment. In four patients tumor progression was not affected by the therapy.

Toxic side effects were similar to those observed in the lymphoma study. The median wbc count nadir was 3400/mm^3 (range, 2600–7000) and the median platelet count nadir was 102,000/mm^3 (range, 26,000–180,000). Two patients had a transient rise in renal function values. In one patient, a grand-mal-like seizure occurred 1 hour after completion of the first *cis*-platinum infusion. Because of tumor progression, no further treatment was administered to this patient. He died 6 weeks later; at necropsy, no brain metastases were found.

Discussion

Although small cell lung cancer is believed to be very responsive to many agents, relatively few new drugs are

TABLE 4.—Characteristics of patients with responding tumors

Patient No.	Age (yrs)	Interval from diagnosis to start of *cis*-platinum therapy (mos)	PS*	Stage†	Prior chemotherapy‡ (response,§ duration)	Response to *cis*-platinum§	Duration of response (mos)	Indicator lesions
1	70	15	1	L	1. A (PR, 7 mos) 2. C (PR, 6 mos)	PR	NE¶	1. Total atelectasis (upper right lobe) 2. Primary tumor
2	50	7	1	L	B (PR, 5 mos)	PR	2.5	Primary tumor
3	70	6	2	L	B (MR, 5 mos)	PR	2.5+	Primary tumor
4	55	9	2	L	A (PR, 8 mos)	PR	NE¶	1. Superior vena cava syndrome 2. Primary tumor
5	56	9	2	E	1. A (PR, 5 mos) 2. C (PR, 3 mos)	MR	2+	1. Primary tumor (regression > 50%) 2. Liver metastases (no change)
6	59	18	3	E	1. A (CR, 15 mos) 2. C (PD)	MR	2 (toxic death)	1. Primary tumor + lung metastases (regression > 75%) 2. Liver metastases (no change)

*PS = performance status (Eastern Cooperative Oncology Group scale).
†E = extensive; L = limited.
‡A = cyclophosphamide, methotrexate, vincristine, and procarbazine. B = cyclophosphamide, methotrexate, vincristine, and VP-16-213. C = Adriamycin and VP-16-213.
§CR = complete remission; PR = partial remission; MR = minor response; PD = progressive disease.
¶NE = duration of remission not evaluable (patient received complementary radiotherapy after achieving PR).

being evaluated in this disease (11). Therefore, it is not surprising that data on the effectiveness of *cis*-platinum in this disease are scanty.

Preliminary results of our ongoing study (four PRs and two clear-cut MRs) suggest a possibly significant antineoplastic activity of *cis*-platinum in small cell lung cancer. At least 14 additional patients must be studied to estimate the degree of the activity, according to recent rules for phase II clinical trials (12).

Our current data are in conflict with those of an unpublished report, which seems to indicate only very moderate antineoplastic activity for *cis*-platinum in this disease.[7] Half of the patients in that unpublished report received < 60 mg/m^2 of *cis*-platinum. All PRs occurring in our trial were noted in patients with limited disease who had a performance status of 1–2. Therefore, differences in patient selection and drug dosage could at least partially account for this inconsistency.

[7]*See* footnote 6.

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Phase II Evaluation of *cis*-Dichlorodiammineplatinum(II) in Lymphomas: A Southwest Oncology Group Study [1]

Arthur H. Rossof, Charles A. Coltman, Jr, Stephen E. Jones, and Robert W. Talley [2,*]

SUMMARY

The Southwest Oncology Group has evaluated the activity of *cis*-dichlorodiammineplatinum(II) at a dose of 75 mg/m^2 given as an iv bolus injection every 3 weeks to 25 fully and partially evaluable patients with advanced Hodgkin's disease and non-Hodgkin's lymphoma. One complete response, two partial responses, and one improvement less than a partial response were noted. Myelosuppression, in the form of leukopenia and thrombocytopenia, was identified and seemed to be more prevalent and more severe than in previous studies. We have attributed this to the extensive prior treatments which these patients had received and to the presence of tumor-bearing marrow which was observed in some of them. The anticipated toxic effects which were noted included nausea and vomiting, anorexia, diarrhea, renal injury, and hyperuricemia. The precise role of *cis*-dichlorodiammineplatinum(II) in the management of human lymphomas awaits elucidation.

[Cancer Treat Rep 63:1605–1608, 1979]

Phase I and II trials of *cis*-dichlorodiammineplatinum(II) (*cis*-platin) have demonstrated its activity in lymphomas (1–5). In 1974, the Southwest Oncology Group initiated a study of this drug in previously treated patients with advanced measurable lymphomas using the dose schedule of 75 mg/m^2 iv every 3 weeks. Four objective responses (one complete response [CR], two partial responses [PRs], and one improvement less than a PR) were seen among the 25 fully and partially evaluable patients.

MATERIALS AND METHODS

Eligibility criteria included the presence of at least one measurable lesion in a patient with histologically verified lymphoma of the Hodgkin's or non-Hodgkin's types, an anticipated survival of ≥ 8 weeks, a complete recovery from toxicity due to previous chemotherapy and/or radiotherapy, a patent urinary tract confirmed by iv pyelography, an absolute granulocyte count of ≥ 2000/μl, a platelet count of ≥ 100,000/μl, a BUN of ≤ 20 mg/dl, and an endogenous creatinine clearance of ≥ 75 ml/minute. All patients provided signed informed-consent forms consistent with the policies of the institution where they were treated and evaluated.

cis-Platin was diluted to a concentration of 1 mg/ml and given as a rapid iv infusion. No effort was made to prehydrate the patients and no forced diuresis with mannitol or furosemide was attempted.

Patients with stable disease or improvement continued to receive the drug at 3-week intervals. Each week, patients had cbcs and platelet counts and measurements of BUN, serum creatinine, and serum uric acid. Before each course of therapy and at the conclusion of the study, a 24-hour endogenous creatinine clearance and an audiogram were assessed.

CR is defined as a complete disappearance of all measurable disease and all disease-related symptoms. PR is defined as a reduction by 50% in the product of the largest perpendicular diameters of each measurable lesion. Improvement is defined as a tumor regression of insufficient magnitude to qualify as PR.

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TABLE 1.—Responses to *cis*-platin among 25 patients with advanced lymphomas

Histopathologic classification*	No. of patients treated	No. of patients with—		
		CR	PR	Improvement
Hodgkin's disease				
Nodular sclerosis	5		1	
Mixed cellularity	2	1		
Unspecified	1			
Non-Hodgkin's lymphomas				
Nodular lymphocytic poorly differentiated	3			
Nodular lymphocytic (unspecified)	1			
Nodular mixed	2			1
Diffuse lymphocytic poorly differentiated	2			
Diffuse mixed	2			
Diffuse histiocytic	6		1	
Diffuse lymphoma (unspecified)	1			

*Histopathologic classifications are according to Lukes (ref 6) (for Hodgkin's disease) and Rappaport (ref 7) (for non-Hodgkin's lymphomas).

TABLE 2.—Toxicity analysis in 25 evaluable patients categorized according to Southwest Oncology Group criteria

Toxicity	Toxicity grade—*					
	1 (mild)	2 (moderate)	3 (severe)	4 (life-threatening)	5 (fatal)	Total
Gastrointestinal						
Nausea and/or vomiting		(11)	(5)			(16)
Diarrhea		(2)				(2)
Renal						
BUN (mg/dl)	21–40	41–60	61–100	> 100		
Creatinine (mg/dl)	1.2–2.0	2.1–4.0	4.1–6.0	> 6.0		
Creatinine clearance (% of pretreatment value)	> 75	50–74	25–49	< 25		
		(3)	(1)			(4)
Hematopoietic						
Wbcs or	3000–3999	2000–2999	1000–1999	< 1000		
polymorphonuclear cells/μl	1001–1500	501–1000	251–500	≤ 250		
	(1)	(3)	(1)			(5)
Platelets/μl	75,000–99,999	50,000–74,999	25,000–49,999	< 25,000		
	(3)		(2)	(4)		(9)

*Nos. in parentheses = No. of patients in whom the given toxicity occurred to the degree indicated.

TABLE 3.—Cumulative data on reported responses of Hodgkin's disease and non-Hodgkin's lymphomas to *cis*-platin (95% confidence limits around the 25.5% figure are 13.9%–40.3%)

Authors (ref No.)	Non-Hodgkin's lymphomas				Hodgkin's disease			
	Total No. of patients	No. of patients with—			Total No. of patients	No. of patients with—		
		CR	PR	Improvement		CR	PR	Improvement
Southwest Oncology Group (this study)	17		1	1	8	1	1	
Rossof et al (1)	2				2	1		
Talley et al (2)	5		3					
Tekuzman et al (3)	3		1					
Hill et al (4)	14		4	2				
Corder et al (5)	6		3					
Totals (% response rates)	47		12 (25.5%)	3	10	2 (20%)	1 (10%)	

RESULTS

Of the 29 patients registered, four are considered unevaluable because of insufficient data (two) or major protocol violations (two). Of the 25 patients evaluated for this report, 16 are fully evaluable for response. The remaining nine patients are considered partially evaluable because they died before receiving the second treatment (four), refused further therapy after the first treatment (three), suffered too much toxicity to justify a second treatment (one), or were lost to followup (one).

Antitumor responses are summarized in table 1.

One patient with mixed cellularity Hodgkin's disease was in CR for 10 weeks. He had received eight other antineoplastic drugs prior to receiving *cis*-platin. The PR in a patient with nodular sclerosis Hodgkin's disease lasted for 6 weeks; this patient had previously received six separate drugs. An 8-week PR occurred in a patient with diffuse histiocytic non-Hodgkin's lymphoma. She had previously been treated with six different drugs. The patient with nodular mixed non-Hodgkin's lymphoma who had improvement had previously been exposed to nine drugs.

Toxicity data are summarized in table 2. These data are similar to those we (1,2) and others have observed previously. The somewhat more severe and commonly noted hematopoietic toxicity appears to be due to the heavy pretreatment characteristics of these patients and to the presence of marrow involvement by tumor which was observed in some of them. The median duration of both leukopenia and thrombocytopenia was 7 days.

Two patients sustained significant increases in the serum uric acid level. Twenty days after receiving *cis*-platin, the serum uric acid of one patient rose from 5.1 to 12.9 mg/dl and this was temporally related to the development of mild renal failure with a BUN value of 31 mg/dl and a serum creatinine value of 3.1 mg/dl. In the other patient, *cis*-platin administration caused an elevation of the serum uric acid from 8.7 to 11.3 mg/dl 7 days later. This level came down to 9.1 mg/dl with allopurinol therapy and was not associated with evidence of renal insufficiency.

DISCUSSION

Out data indicate that *cis*-platin has some activity against advanced lymphomas, even in those patients with extensive prior chemotherapy. The results of this clinical trial are pooled in table 3 with data from five earlier studies in which patients with non-Hodgkin's lymphomas or Hodgkin's disease were treated with *cis*-platin. Some of these trials were phase I studies with patients receiving *cis*-platin in varying doses (1,2). It is possible that response rates would have been higher if patients had received higher doses or if they had been treated earlier in their clinical courses.

The most common and unpleasant side effects of *cis*-platin are the nausea and vomiting and protracted anorexia that the drug can cause. In addition, a greater degree of hematopoietic toxicity was seen in this group of heavily pretreated patients, some with marrow involvement by tumor.

The ultimate role of *cis*-platin in the management of lymphomas has not yet been defined. It is possible that drug combinations which include *cis*-platin at a lower dose will prove to be of value.

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cis-Dichlorodiammineplatinum(II) in Adult Patients: Southwest Oncology Group Studies[1]

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SUMMARY

In addition to *cis*-dichlorodiammineplatinum(II) studies in lymphomas and testicular cancer and a broader phase II study of all genitourinary malignancies, the Southwest Oncology Group is currently conducting eight other studies which examine the effectiveness of this drug in adult patients with a variety of tumor types. This report will give preliminary analyses of the five studies for which there are early data, plus briefly describe the design of the three remaining protocols for which there are no data as yet.

[Cancer Treat Rep 63:1609–1610, 1979]

MATERIALS AND METHODS

In the studies reported in this paper, responses are defined as they are in most Southwest Oncology Group (SWOG) protocols. A complete response (CR) is defined as the total disappearance of measurable tumor which lasts for ≥ 1 month. A partial response (PR) is defined as a ≥ 50% reduction in measurable tumor which lasts for ≥ 1 month and occurs in a clinical setting where no new lesions appear. In those studies which include patients with multiple myeloma, a given percent decrease in the M protein is utilized to define response.

Toxicity is still the subject of ongoing analyses in these studies. Preliminary evaluations by each protocol coordinator suggest acceptable myelosuppression in most studies, and an early assessment of nephrotoxicity has been rendered by the protocol chairman.

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RESULTS

SWOG-7611

In a previously treated group of patients with sarcoma, Samson evaluated *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) at a dose schedule of 15 mg/m^2 daily × 5 every 28 days. In 42 patients with soft tissue sarcoma, there were three partial responders and no complete responders (table 1). In 12 patients with bony sarcomas (primarily osteogenic sarcoma) there were no responders. One of seven patients achieved a CR in the category of "other" sarcoma (primarily mesothelioma).

Hematologic toxicity was judged to be acceptable; only 14% of the evaluable treatment courses were associated with a wbc count of < 3000/mm^3 and only 24% of the evaluable treatment courses were associated with a platelet count of < 100,000/mm^3. Nausea and vomiting were observed in 66% of the patients treated. Posttreatment

TABLE 1.—SWOG-7611: sarcomas (*cis*-platinum at a dose of 15 mg/m^2 daily × 5 q28days)

Sarcoma	No. of patients	No. of responses		%
		PR	CR	PR + CR
Soft tissue	42	3	0	7
Bony	12	0	0	0
Other	7	0	1	14

serum creatinine levels exceeded 2.0 mg/dl in only four patients and exceeded 4.0 mg/dl in only one patient.

SWOG-7706

Alberts examined *cis*-platinum combination chemotherapy regimens in previously treated patients with ovarian cancer using *cis*-platinum (at a dose schedule of 50 mg/m^2 iv q28days) in combination with 5-fluorouracil and hexamethylmelamine (three-drug combination) or all three drugs plus Adriamycin (four-drug combination). Patients receiving the three-drug combination had previously failed to respond to Adriamycin and an alkylating agent, whereas those patients treated with the four-drug combination had previously failed to respond only to an alkylating agent.

Of the 27 patients receiving the three-drug combination, three achieved a PR and none achieved a CR (table 2). Of 14 evaluable patients receiving the four-drug combination, six achieved a PR and one achieved a CR (PR + CR, 50%).

No drug-related deaths were recorded, although thrombocytopenia was severe or life-threatening in 32% of those patients receiving the three-drug combination and in 25% of those receiving the four-drug combination. Nephrotoxicity was not severe or prolonged in this group of patients. One patient developed moderate paresthesias in the lower extremities.

SWOG-7629

cis-Platinum, at a dose schedule of 50 mg/m^2 iv on Days 1 and 8 of each 28-day cycle, was used by Panettiere in the study of head and neck cancer. Of 48 evaluable patients, all previously treated with surgery, irradiation, and chemotherapy, ten had PRs and three had CRs (PR + CR, 27%).

Hematologic toxicity was modest; nausea and vomiting occurred in 85% of the patients but were considered severe in only 15%. Fifteen of the 48 patients experienced elevations of BUN (> 30 mg/dl) and/or serum creatinine (> 2.0 mg/dl) levels. Only two patients experienced severe renal failure; it occurred in one patient starting with the first dose of *cis*-platinum and in the other patient after the second course.

TABLE 2.—SWOG-7706: ovarian cancer (*cis*-platinum, at a dose of 50 mg/m^2 q28days, plus 5-fluorouracil and hexamethylmelamine with or without Adriamycin)

Combination	No. of patients	No. of responses		%
		PR	CR	PR + CR
3-drug	27	3	0	11
4-drug	14	6	1	50

SWOG-7719

Bonnet used a 4-day (Days 2–5) program of *cis*-platinum at a dose of 20 mg daily every 21 days in a study of patients with alkylating agent-resistant multiple myeloma. *cis*-Platinum was used in combination with Day-1 administration (of each 21-day cycle) of bleomycin, vincristine, BCNU, Adriamycin, and prednisone. Four of 16 evaluable patients achieved an objective response as measured by a 50% decrease in the M protein. One of these four patients achieved a > 75% decrease in the M protein.

Toxicity is still the subject of ongoing evaluation in this otherwise very early analysis. Preliminary evaluation by the protocol coordinator suggests that the combination program is no better than other second-line treatment for multiple myeloma, and that the 5-day iv program every 21 days is a major inconvenience to the patient.

SWOG-7730

Melanoma patients (with prior chemotherapy) are the subject of Al-Sarraf's randomized trial of *cis*-platinum (100 mg/m^2 every 21 days with iv hydration) versus *cis*-platinum, hydration, and mannitol. Only 14 patients are evaluable, or partially evaluable, and thus far no patient has responded. Renal toxicity has been graded moderate to life-threatening in three patients, and one drug-related death is thought to have occurred.

Other SWOG Studies

Three other *cis*-platinum studies have been recently started in the SWOG. In each instance it is too early to evaluate response and toxicity, and the schedules are simply outlined here. Panettiere will be examining this drug at a dose of 50 mg/m^2 on Days 1 and 8 of each 28-day cycle in two separate studies of patients with esophageal and lung cancers. The third study by Lehane, utilizing the same schedule, will evaluate *cis*-platinum in patients with head and neck cancer, comparing this drug to methotrexate.

DISCUSSION

Although some disease categories contain small numbers of patients, the following limited interpretations seem justified. *cis*-Platinum may have sufficient activity in Hodgkin's disease to justify further study. In alkylating agent-resistant patients with ovarian cancer, the compound in combination with other drugs is active and deserves serious consideration as second-line therapy. The 27% response rate in heavily pretreated patients with head and neck cancer is sufficiently positive to warrant additional investigation in epithelial malignancies.

The relative inactivity of *cis*-platinum in our studies of non-Hodgkin's lymphoma, multiple myeloma, and sarcomas has been less encouraging.

Evaluation of *cis*-Dichlorodiammineplatinum(II) in Children With Advanced Malignant Diseases: Southwest Oncology Group Studies [1]

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SUMMARY

Two schedules of *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) were evaluated for therapeutic efficacy and toxicity in children with malignant diseases resistant to standard therapy. Initially, *cis*-platinum was given as a rapid iv bolus injection at a dose of 15 mg/m^2/day for 5 days every 3 weeks. The second schedule of *cis*-platinum was a dose of 1 mg/kg/week administered as an 8-hour infusion with mannitol, furosemide, and hydrating fluids. Using the daily schedule, no responses were seen among 23 children with acute lymphatic leukemia and only eight responses were noted among 47 children with solid tumors. Using the weekly schedule, three responses were noted among 25 children with solid tumors. Responses were observed in seven children with neuroblastoma, two with osteosarcoma, one with embryonal testicular carcinoma, and one with an endodermal sinus tumor. With one exception (a 4-year-old child with neuroblastoma), all responses were of short duration. The most common side effects with both schedules were nausea and vomiting which were usually controlled with antiemetics. The dose-limiting toxicity, especially on the 5-day schedule, was renal function impairment. Only one child who received *cis*-platinum weekly as an 8-hour infusion with diuresis had elevation of the serum creatinine level. Protocols are being initiated to determine the therapeutic effectiveness and toxicity of combination therapy with *cis*-platinum in children with neuroblastoma and osteosarcoma.

[Cancer Treat Rep 63:1611–1614, 1979]

cis-Dichlorodiammineplatinum(II) (*cis*-platinum) is an inorganic compound with proven antitumor activity in a variety of animal and human malignancies (1–3). Although numerous in vivo and in vitro studies have been done, an optimal schedule has yet to be defined. One clinical study suggested that weekly doses of the agent administered by infusion over 6–8 hours with adequate diuresis are superior to daily doses without induced diuresis (4). The principal acute dose-limiting toxic effects observed were nausea, vomiting, and nephrotoxicity; nephrotoxicity and myelotoxicity limited long-term administration of the agent (2,3,5,6). Hearing impairment was also a disturbing side effect. The following summarizes the results seen in 109 children treated in the Southwest Oncology Group (SWOG) studies which began in October 1973 to determine the toxicity and therapeutic efficacy of *cis*-platinum in the treatment of solid tumors and acute lymphatic leukemia.

MATERIALS AND METHODS

If resistant to standard therapy, children with acute lymphatic leukemia in bone marrow relapse or children

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with measurable solid tumor lesions with evidence of progressive disease were eligible for these studies, providing they had normal renal function. Informed consent according to institutional policy was obtained for all patients.

The initial schedule of *cis*-platinum was 15 mg/m^2/day for 5 days by rapid iv bolus injection every 3 weeks. Provisions were made for escalation of the dose to 20 mg/m^2 if undue toxicity was not observed. No special precautions were taken for adequate hydration or control of emesis. In June 1976, the dose of *cis*-platinum was changed to 1 mg/kg to be administered weekly as an 8-hour infusion with furosemide, mannitol, and hydrating fluids to induce diuresis. All patients were pretreated with an antiemetic. Urine output was regulated to 5 ml/kg/hour. Entry in this latter study was limited to children with solid tumors.

Prestudy evaluation included documentation of prior therapy, physical examination, radiographic determination of extent of disease, and routine hemogram, urinalysis, serum electrolyte, and hepatic and renal function studies. A bone marrow aspiration was required for all children with acute leukemia. Auditory and vestibular function studies were optional. Hemogram, urinalysis, serum BUN, creatinine, calcium, magnesium (optional), and SGOT levels were to be obtained weekly and vestibular and auditory studies were to be obtained every 3 weeks. Measurement of serum electrolyte levels was required at 8 and 24 hours for patients receiving the weekly schedule. Antiemetics were administered as needed for control of vomiting. Provisions for reduction of dose and/or delay of therapy were provided in case of excessive nephrotoxicity or myelotoxicity. To determine response to therapy, every 3 weeks a bone marrow aspirate was required for children with acute lymphatic leukemia and measurement of the lesions either by physical examination and/or radiographic studies was required for children with solid tumors.

The criteria for response in children with acute lymphatic leukemia were based on reduction of blast cells in the bone marrow aspirate and were defined as: *complete remission*—$\leq$ 5% blast cells; *partial remission*—$\geq$ 5% to $\leq$ 25% blast cells; and *no response*—$>$ 25% blast cells. In children with solid tumors, response to therapy was defined as: *complete response*—clinical disappearance of all lesions; *partial response*—clinical regression of all lesions by $\geq$ 50%; and *improvement*—clinical regression of the total tumor size to $\leq$ 50% (one or several metastatic lesions may show a $>$ 50% reduction in size). Children who died after $<$ 1 week in the study were not evaluable, and children who died after $<$ 2 weeks in the study were evaluable for toxicity but not for response.

RESULTS

The response to therapy for children with acute lymphatic leukemia and solid tumors receiving *cis*-platinum at a dose of 15 mg/m^2/day for 5 days is shown in table 1. One child with acute lymphatic leukemia died after three doses of therapy. There was no change in the bone marrow status in 23 children with acute lymphatic leukemia although there was a transient decrease in the liver and spleen size in three children. Seven children with solid tumors were not evaluated for response: one child died on the fourth day and six died within 2 weeks of entry in the study. Objective responses were noted in eight of 47 children. The child with the complete response had marrow infiltration with neuroblastoma cells which cleared after two courses of therapy. After nine courses (Day 245), therapy was stopped and he had recurrence of his disease shortly thereafter. Three children with neuroblastoma and one child with osteosarcoma had a partial

TABLE 1.—Response to therapy with *cis*-platinum (15 mg/m^2/day for 5 days every 3 wks)

Disease	No. of responses*					Duration of responses in days
	CR	PR	IMP	NR	Totals	
Acute lymphatic leukemia	0	0	0	23	23	
Neuroblastoma	1	3	2	17	23	245 (CR); 45, 58, 113 (PR); 132, 120 (IMP)
Ewing's sarcoma	0	0	0	3	3	
Rhabdomyosarcoma	0	0	0	6	6	
Osteosarcoma	0	1	0	0	1	80 (PR)
Testicular embryonal carcinoma	0	0	1	1	2	88 (IMP)
Retinoblastoma	0	0	0	2	2	
Miscellaneous tumors	0	0	0	10	10	

*CR = complete response; PR = partial response; IMP = improvement but less than a partial response; NR = no response.

TABLE 2.—Response to therapy with *cis*-platinum (1 mg/kg/wk with osmotic diuresis)

Disease	No. of responses*					Duration of responses in days
	CR	PR	IMP	NR	Totals	
Neuroblastoma	0	0	1	4	5	64 (IMP)
Ewing's sarcoma	0	0	0	8	8	
Osteosarcoma	0	1	0	0	1	164 (PR)
Miscellaneous tumors	0	0	0	9	9	

*CR = complete response; PR = partial response; IMP = improvement but less than a partial response; NR = no response.

response, and two children with neuroblastoma and one child with testicular embryonal carcinoma had improvement of their disease.

Thirty-one children with solid tumors received 1 mg/kg of *cis*-platinum weekly along with a diuretic regimen. There were two early deaths and four children died within 2 weeks of entry in the study. Of 25 children evaluable for response, one child with neuroblastoma and one child with an endodermal sinus tumor had improvement of disease, and one child with osteosarcoma had a partial response (table 2).

TOXICITY

Nausea and vomiting were the most common side effects observed for both schedules of *cis*-platinum; in most cases they were effectively controlled with antiemetics. Emesis more than two times daily on one or more occasions occurred in 17 of the 76 evaluable patients. One child vomited more than six times in a 24-hour period. Emesis was more frequent and severe on the weekly schedule. There were 29 evaluable children; 19 had emesis which was not controlled by pretreatment with antiemetics and three had emesis which occurred more than six times.

Laboratory evaluation of the children receiving the daily schedule revealed that serum creatinine and BUN levels were elevated in 14 children at time intervals varying between 3 and 46 days (median, 24) and 5 and 92 days (median, 47) respectively. The duration of elevated serum BUN and creatinine levels ranged between 1 and 20 days (median, 9). Two of the three children who had elevated serum creatinine levels at the start of therapy had normal values 6 days after the last dose of *cis*-platinum; however, transient elevation occurred with subsequent courses. The third child who died of progressive disease on Day 13 had continuous elevation of serum creatinine levels until the time of death. Two of these children developed hypocalcemia while receiving treatment.

Only one of the 29 children evaluable for toxicity had an elevation of the serum creatinine level to 1.3 mg/dl on the weekly schedule with the diuresis regimen. There were significant changes in the serum electrolyte levels at the completion of the 8-hour diuresis regimen but these were not associated with any clinical symptoms. By 24 hours, all values had returned to the initial baseline levels.

Two children who were receiving the 5-day daily dose schedule had asymptomatic depression of the serum magnesium levels. One child had a serum magnesium level of 0.43 mg/dl on Day 28 with normal serum creatinine and calcium levels, but 2 days after the second course the serum calcium level decreased to 7 mg/dl. On Day 48, the serum creatinine level was elevated to 2.7 mg/dl, the serum calcium level was 9.1 mg/dl, and the serum magnesium level was 1.9 mg/dl. On Day 104, the serum magnesium level in the other child was 1.2 mg/dl.

Thirty-nine children who received two or more 5-day courses of *cis*-platinum and who had initial normal hemoglobin values and/or platelet counts and/or wbc counts were evaluated for the myelosuppressive effect of the agent. Ten of the 11 children with pretreatment hemoglobin levels > 10 g/dl had a decrease of the hemoglobin level to 6.8–9.9 g/dl. Thrombocytopenia (20,000–83,000 platelets/μl) occurred in ten of the 31 children with pretreatment platelet counts > 100,000/μl. Six of 29 children with pretreatment absolute neutrophil counts > 1000/μl had counts between 480 and 850/μl during therapy. Evidence of myelosuppression was transient and occurred within a median of 42 days (range, 21–85). Therapy was delayed for 1 and 3 weeks in two patients, but the total dose was not altered.

Preliminary analysis of the myelosuppressive effect of the weekly schedule revealed development of thrombocytopenia in four of 19 children. Only one of 29 children had a depression of the granulocyte count.

FUTURE STUDIES

With the demonstration that *cis*-platinum has therapeutic efficacy in the treatment of children with advanced metastatic osteosarcoma and neuroblastoma resistant to known effective agents, the SWOG is designing studies to evaluate the efficacy of *cis*-platinum when used in combination with other known effective agents. A phase II

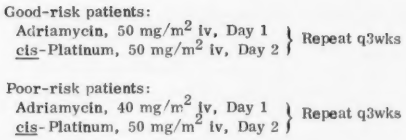
Good-risk patients:
Adriamycin, 50 mg/m2 iv, Day 1
cis-Platinum, 50 mg/m2 iv, Day 2
Repeat q3wks
Poor-risk patients:
Adriamycin, 40 mg/m2 iv, Day 1
cis-Platinum, 50 mg/m2 iv, Day 2
Repeat q3wks

FIGURE 1.—Regimen for phase II study of Adriamycin and *cis*-platinum in children with metastatic neuroblastoma resistant to standard therapy. *cis*-Platinum is given as an 8-hr infusion with mannitol, furosemide, and water diuresis after pretreatment with antiemetics.

protocol to determine the efficacy of Adriamycin plus *cis*-platinum when used in combination for the treatment of children with neuroblastoma resistant to vincristine and cyclophosphamide has been activated and is admitting patients. The therapeutic regimen is shown in figure 1. A pilot protocol for use in the initial treatment of children with neuroblastoma with advanced disease is under consideration and involves a five-drug combination of vincristine, cyclophosphamide, BCNU, Adriamycin, and *cis*-platinum. This pilot study will determine the toxicity of this regimen and accrue preliminary data as to its therapeutic efficacy.

Since 5-fluorouracil has been shown in previous SWOG studies to have antitumor effectiveness in patients with advanced metastatic osteosarcoma (7), a phase I study has been designed to determine the toxicity of 5-fluorouracil and *cis*-platinum when used in combination. Once the maximum-tolerated dose levels and toxicity of the two agents have been determined, it is proposed to evaluate this drug combination for therapeutic efficacy in children with metastatic osteosarcoma resistant to methotrexate and Adriamycin.

DISCUSSION

This study has demonstrated that *cis*-platinum has an antitumor effect in children with neuroblastoma, osteosarcoma, embryonal carcinoma, and endodermal sinus tumor, but has no effect in children with acute lymphatic leukemia. The responses have been relatively minimal and of short duration for the most part, but all of the children entered in the study had advanced disease resistant to multiple, known effective agents. The response rate for the weekly schedule appeared similar to the response rate for the daily schedule. In those children who responded to therapy, the response usually occurred within 3 weeks after the 5-day course or after three weekly courses of the drug, but usually was not maximal until after the second course of the sixth week.

The principal dose-limiting toxic effect was nephrotoxicity which was reversible with cessation of therapy. It is not clear whether hydration and diuresis decrease the toxicity, but the children who received a diuretic regimen with the weekly therapy seemed to have less renal toxicity, even though the dose was larger. Myelotoxicity was minimal, requiring delay of therapy in only two children. No reduction in dose was necessary.

Further studies are planned with *cis*-platinum in combination with other known effective agents.

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cis-Dichlorodiammineplatinum(II) in Childhood Cancer[1]

Arnold I. Freeman,* Lawrence J. Ettinger, and Martin L. Brecher[2]

SUMMARY

***cis*-Dichlorodiammineplatinum(II) (*cis*-platinum) was evaluated in three separate studies at Roswell Park Memorial Institute in children and adolescents with cancer. In the first study, 16 patients with a variety of solid tumors were treated. Objective responses were seen in patients with neuroblastoma, osteogenic sarcoma, seminoma, and medullary carcinoma of the thyroid. In the second study, five of eight patients with far-advanced osteogenic sarcoma showed objective responses to *cis*-platinum. In the third study, *cis*-platinum and Adriamycin were employed as primary adjuvant chemotherapy along with surgery in osteogenic sarcoma. Nine of ten patients have remained disease-free from 8 to 31 months (mean, 19 months). *cis*-Platinum is an active agent in pediatric tumors.**

[Cancer Treat Rep 63:1615–1620, 1979]

cis-Dichlorodiammineplatinum(II) (*cis*-platinum) is a chelated heavy metal with antitumor properties. It is thought to act as an alkylating agent (1). This compound was initially isolated by Rosenberg in 1965 who noted inhibition of *Escherichia coli* replication. Antitumor properties were discovered, and studies in animal models showed a wide range of responsiveness in chemically and virally induced transplantable tumor systems (2,3). In man, a wide range of antitumor activity was identified, notably against testicular carcinomas and epidermal carcinomas of the head and neck (4–8). There are, however, a few reports of this drug's efficacy in childhood cancer; the present report deals with the experience at Roswell Park Memorial Institute in childhood cancer.

MATERIALS AND METHODS

Three separate groups of patients were evaluated.

Group I.—Sixteen patients with far-advanced cancer refractory to conventional chemotherapy were given *cis*-platinum in a variety of dose schedules. There were 11 males and five females ranging in age from 2 to 16 years. Courses of therapy were continued until dose-limiting toxicity appeared (primarily renal) or until tumor progression was documented.

Group II.—Nine patients with far-advanced osteogenic sarcoma ranging in age from 13 to 43 years received *cis*-platinum. All patients had received prior adjuvant Adriamycin (ADR) and six patients had received high-dose methotrexate prior to *cis*-platinum; four patients had received other additional chemotherapy. All patients had advanced and clearly progressive disease before *cis*-platinum was employed.

Group III.—Ten consecutive patients with a diagnosis of osteogenic sarcoma were treated with primary adjuvant ADR and *cis*-platinum after surgery from January 1976 through December 1977. The patients' ages ranged from 13 to 46 years, with eight of ten patients between the ages of 13 and 20 years. There were seven males and three females. The primary lesions were located in the proximal tibia (two patients), proximal humerus (one), distal femur (five), and proximal femur (one). One patient, a 16-year-old white male, developed an osteogenic sarcoma in the radiation port 14 years after the diagnosis of bilateral retinoblastoma; as an infant he had received 8000 rads in two courses given in an attempt to save his less-involved left eye. The pathologic specimen was consistent with osteogenic sarcoma "ordinaire" in all patients but one, in whom a telangiectatic pattern was present. Preoperative evaluation to rule out metastatic disease included a skeletal survey and/or bone scan, a chest roentgenogram, and a chest tomogram in all patients. No patients had evidence of metastatic disease at diagnosis. Surgery consisted of amputation (seven patients), hem-

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ipelvectomy (one), limb salvage (one), and local resection of the orbital bone (one). In the latter patient, all gross tumor was surgically removed by a wide excision, and the osteogenic sarcoma was found to have invaded the dura and the region of the cribriform fossa. Histologic evaluation showed that the tumor extended to the line of resection. Following amputation, adjuvant chemotherapy was instituted with ADR and *cis*-platinum. Courses of ADR at 30 mg/m^2/day × three doses and *cis*-platinum at 100 mg/m^2 were alternated every 3 weeks until a maximum dose of 540 mg/m^2 of ADR was administered. *cis*-Platinum was to be continued every 4 weeks to complete 1 year of chemotherapy.

cis-Platinum was administered in various routes and schedules. In the first study, lower doses were used initially and given daily. Recently, *cis*-platinum has been given at a dose of 100 mg/m^2 as an iv infusion over 4 hours. Forced iv fluid diuresis was begun 1–2 hours prior to *cis*-platinum and continued for 24 hours. All patients received mannitol with the *cis*-platinum infusion.

When *cis*-platinum is given alone in far-advanced disease, it is given weekly or biweekly. In primary adjuvant chemotherapy for osteosarcoma, it is given in a cyclic fashion every 6 weeks alternating with ADR (fig 1).

Definition of Response

A complete response (CR) was defined as a disappearance of all measurable parameters of disease, a partial response (PR) was defined as a reduction by ≥ 50% in the volume of measurable tumor, and improvement was defined as a decrease in volume by < 50% or a definite improvement of symptomatic status. No response was defined as no visible negative or positive change and progressive disease was defined as an increase in the size of the lesion, with or without clinical deterioration.

Complete medical history and physical examination were performed for each patient at each treatment session and at regular intervals after chemotherapy was completed. In addition to routine chemistries and cbcs, creatinine clearance determinations, chest roentgenograms, chest tomograms, bone scans and/or skeletal surveys, surgical stump roentgenograms, electrocardiograms, urinalyses, and audiograms were obtained on a regular basis in most patients during and after the completion of therapy.

Informed consent was obtained from all patients and/or guardians prior to entry in this study.

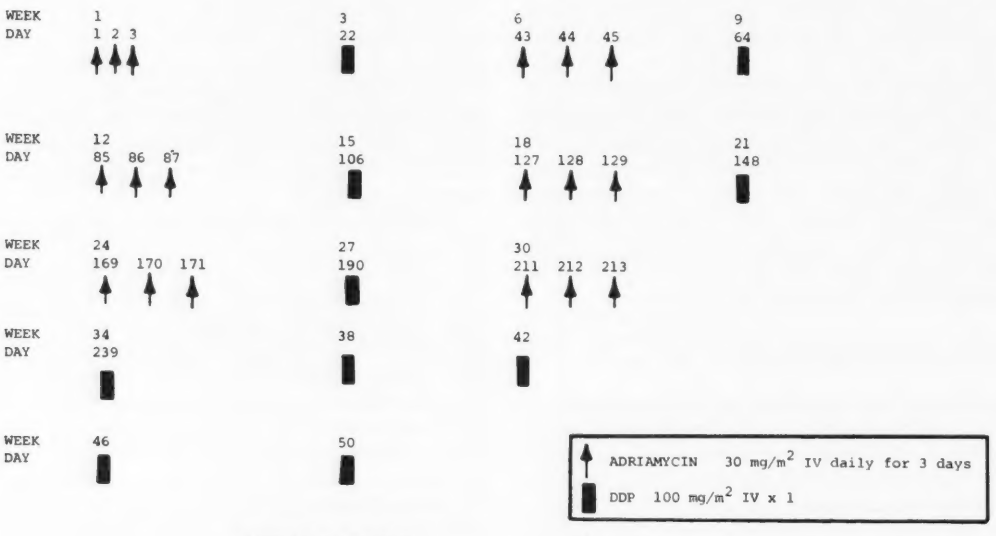

FIGURE 1.—Schema for primary adjuvant chemotherapy for osteogenic sarcoma.

RESULTS

No CRs were noted in the first study (table 1) in children with far-advanced cancer of various types. PRs were seen in one patient with osteogenic sarcoma, in one with extragonadal seminoma, in one with medullary carcinoma of the thyroid, and in one with neuroblastoma. Two other patients with neuroblastoma showed improvement.

In the second study (table 2) in patients with advanced osteogenic sarcoma, one patient had a CR with complete clearing of a large left pulmonary mass and pleural effusion and she remains disease-free as determined by roentgenograms and tomograms for > 20 months. Three patients with pulmonary metastases had PRs lasting 2–5 months, and a fourth patient had a PR with regression of bony metastases for 5 months. A teenage girl had a local recurrence in the mandible after subtotal mandibular resection and 10 months of adjuvant ADR therapy; she subsequently underwent further resection and received 320 mg/m^2 of *cis*-platinum over a 4-month period and is currently disease-free for > 53 months.

In the third study (table 3) primary adjuvant ADR and *cis*-platinum were utilized in osteosarcoma; all ten patients are alive, nine without evidence of disease. A single patient has had a local recurrence without distant metastases. The nine disease-free patients had extremity lesions with either amputation (eight) or a wide excision with a limb salvage procedure (one). The mean disease-free interval from diagnosis is 19 months (range, 8+–31+ months). Seven of the nine patients who are disease-free have not received therapy from 5+ to 23+ months (mean, 13 months). Only one patient had disease recurrence (20 months after diagnosis and 11 months after chemotherapy was discontinued). This patient had probable radiation-induced orbital osteogenic sarcoma with microscopically incomplete resection.

Toxicity

Toxicity was similar to that previously described (9–11). Nausea and vomiting was universal and profound and was most noticeable on the day of drug administration but occasionally lasted 1 or 2 days longer. Transient alterations of renal function tests were common and were seen in approximately one half of the patients. It appears that with more widely spaced *cis*-platinum administration, forced diuresis, and administration of drug over a 4-hour period, we are seeing less renal toxicity than previously observed. Permanent and moderate renal impairment was noted in two patients, both with far-advanced disease: one osteogenic sarcoma and one medullary carcinoma of the thyroid. The latter patient still has moderately impaired renal function 3 years after *cis*-platinum (BUN levels ranging between 50 and 60 mg/100 ml). Bone marrow depression was mild.

Transient elevation of liver enzymes, ie, transaminase, was noted in approximately 35% of patients.

Tinnitus and high-frequency hearing loss in audiograms were noted in approximately 40% of patients. Following cessation of *cis*-platinum, tinnitus has disappeared and hearing function has improved or returned to normal.

There appeared to be a transient elevation of blood pressure with forced fluid diuresis accompanying *cis*-platinum administration in several patients after repeated administration of *cis*-platinum. There was one case of peripheral neuropathy (11).

Finally, there was an apparent "platinum line" in the

TABLE 1.—Group I study: response to *cis*-platinum in patients with far-advanced cancers of various types

Neoplasm	No. of patients treated	No. of patients with—				
		CR	PR	Improvement	No response	Progressive disease
Osteogenic sarcoma	4		1		1	1
Ewing's sarcoma	2				2	
Rhabdomyosarcoma	2				1	1
Neuroblastoma	3		1	2		
Seminoma	1		1			
Medullary carcinoma of thyroid	1		1			
Undifferentiated carcinoma of nasopharynx	1				1	
Wilms' tumor	1				1	
Acute lymphocytic leukemia	1				1	

TABLE 2.—Group II study: response to *cis*-platinum in patients with far-advanced osteosarcoma

Patient No.	Age/ sex	Primary tumor site	Surgery	Prior chemotherapy* and clinical course	Response to *cis*-platinum
1	14/F	Left mandible	Left mandibulectomy	Adjuvant ADR, local recurrence	Disease-free for 53 mos
2	13/F	Left proximal humerus	None	ADR + HDMTX for pulmonary metastases at presentation, progressive disease	None
3	13/M	Left distal femur	Mid-femur amputation	Adjuvant ADR for pulmonary metastases; autologous tumor cells and ifosfamide, progressive disease	None
4	19/M	Left distal tibia	High-thigh amputation	Adjuvant ADR for pulmonary metastases	PR, 75% shrinkage of skull metastases
5	15/M	Left distal femur	Initial tumor resection and bone graft; left hip disarticulation 3 wks later	Adjuvant ADR for pulmonary metastases	None
6	22/M	Left distal femur	Mid-femur amputation	Adjuvant HDMTX, ADR, and VCR for pulmonary metastases; CYC, no response	PR, 50% shrinkage of pulmonary metastases
7	14/F	Left proximal tibia	High-femur amputation	Adjuvant ADR and HDMTX for pulmonary metastases	PR, 50% shrinkage of pulmonary metastases
8	18/F	Right distal femur	High-femur amputation	Adjuvant ADR for pulmonary metastases	CR, complete clearance of pulmonary metastases for 20 mos
9	43/F	Left femur	Left hip disarticulation	Pulmonary metastases 20 mos after surgery; CYC, VCR, ADR, and HDMTX, no response	PR, 50% shrinkage of pulmonary metastases

*HDMTX = high-dose methotrexate; VCR = vincristine; CYC = cyclophosphamide.

gingivae without symptomatology noted in one patient on three occasions soon after *cis*-platinum administration.

DISCUSSION

The mechanism of action of *cis*-platinum is similar to the action of a bifunctional alkylating agent; it binds preferentially, but not exclusively, to guanine (1,12–15). Rosenberg (13) also postulates that *cis*-platinum enhances the antigenicity to the tumor system. At low concentrations, in vitro *cis*-platinum selectively inhibits RNA and protein synthesis.

cis-Platinum is most effective when given iv and is widely distributed, with < 10% of the *cis*-platinum remaining in the plasma 1 hour after administration (16). It is first concentrated in the kidney and then in the liver (17). Clearance is biphasic with an initial half-life of 30–50 minutes and a second half-life of 2–5 days (6). *cis*-Platinum has a low therapeutic index (18). The drug is excreted in the urine.

cis-Platinum has a wide spectrum of tumor efficacy in childhood cancer. In our initial study, it demonstrated efficacy in neuroblastoma, seminoma, medullary carcinoma of the thyroid, and osteogenic sarcoma. Nitschke et al (19), for the Southwest Oncology Group, have also noted the efficacy of *cis*-platinum in a spectrum of childhood tumors. They observed objective responses in four of 25 patients with neuroblastoma, in one of two with embryonal cell carcinoma of the testes, and in one of one with osteosarcoma. The responses in neuroblastoma and osteogenic sarcoma are of particular interest in the childhood and adolescent period.

In our second study, we confirmed the efficacy of *cis*-platinum in osteogenic sarcoma and noted that > 50% of patients had objective responses. One patient with a large pulmonary metastasis and pleural effusion had a CR and remains disease-free 20 months after *cis*-platinum therapy.

Approximately 2½ years ago, we initiated a program of primary adjuvant ADR and *cis*-platinum with surgery

TABLE 3.—Group III study: primary adjuvant *cis*-platinum + ADR in patients with osteosarcoma

Patient No.	Age/ sex	Primary tumor site	Date (mo/yr) of diagnosis	Surgery	Total dose (mg/m²) of—		Date (mo/yr) of last chemotherapy
					ADR	*cis*-Platinum	
1	16/M	Left distal femur	1/76	High above-knee amputation	540	180	9/76
2	19/M	Right distal femur	5/76	High above-knee amputation	540	800	5/77
3	13/F	Right distal femur	9/76	High above-knee amputation	540	800	8/77
4	13/F	Left proximal femur	10/76	Hemipelvectomy	510	400	5/77
5	16/M	Left orbit	10/76	Wide excision; histologically, tumor extended to line of resection	540	500	7/77
6	14/M	Left distal femur	11/76	High above-knee amputation	490	600	8/77
7	46/M	Right distal femur	7/77	High above-knee amputation	450	480	1/78
8	17/F	Right proximal tibia	7/77	Mid-thigh amputation	520	620	4/78
9	29/M	Right proximal tibia	11/77	Mid-thigh amputation	Still receiving therapy		
10	20/M	Right proximal humerus	12/77	Wide excision	Still receiving therapy		

in newly diagnosed patients with osteosarcoma. *cis*-Platinum and ADR have been used in combination in studies of patients with advanced solid tumors (20–22). No potentiation of toxicity occurred and it has been suggested that this combination is synergistic. This combination appears very attractive in osteogenic sarcoma where ADR is also an effective agent and where the primary toxic effects do not overlap (ie, bone marrow depression and cardiotoxicity from ADR and renal and auditory toxicity from *cis*-platinum).

Nine of ten patients receiving this program of adjuvant chemotherapy remain disease-free from 8 to 31 months (mean, 19 months). The single patient who relapsed probably had a radiation-induced osteogenic sarcoma with microscopically incomplete resection of the tumor.

Renal toxicity has been the major dose-limiting problem with the use of *cis*-platinum (2,23). It is cumulative, but usually initially reversible. Toxicity has been initially manifested by decreasing creatinine clearances followed by elevated serum urea nitrogen and creatinine and by proteinuria. The pathogenesis appears to be acute tubular necrosis similar to kidney damage induced by heavy-metal poisoning (2). In our studies, two patients had irreversible kidney damage.

The other significant irreversible toxicity that has been reported has been bilateral ototoxicity which progresses from higher to lower frequencies. Initially, this is not recognized by the patients but is detected by serial audiograms. Eight patients in our three series showed mild high-frequency loss.

In our studies, gastrointestinal symptoms of anorexia and nausea and vomiting occurred in all patients and were generally severe.

Bone marrow suppression occurred, but was usually mild. Hemoglobin levels remained generally stable and wbc and platelet counts rarely fell below 2000/mm^3 and 100,000/mm^3, respectively. The nadir occurred between 14 and 21 days.

Transient hepatotoxicity was also noted in our patients. Serum transaminase elevation has also been mentioned in several studies (6).

In summary, *cis*-platinum appears to be an effective agent in childhood solid tumors. However, the amount of drug that can be administered is limited because of toxicity, particularly the renal toxicity and ototoxicity.

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Phase II Study of *cis*-Dichlorodiammineplatinum(II) in Childhood Osteosarcoma: Children's Cancer Study Group Report

Edward S. Baum, Paul Gaynon, Lowell Greenberg, William Krivit, and Denman Hammond [1,*]

cis-Dichlorodiammineplatinum(II) (*cis*-platinum) acts as a bifunctional alkylating agent with preferential binding to guanine (1,2). However, interstrand cross-linking cannot account for the degree of observed DNA inactivation (3,4).

The Division of Cancer Treatment of the National Cancer Institute (Bethesda, Md) has sponsored *cis*-platinum as an investigational agent since 1972 (1). *cis*-Platinum has demonstrated activity against carcinomas of the ovary (5), bladder (6), and head and neck (7). It is among the most active agents in embryonal carcinoma of the testicle (8,9).

cis-Platinum has demonstrated activity in several childhood malignancies. Kamalakar et al (10) reported four objective responses among 16 children with advanced cancer refractory to conventional therapy, one each in neuroblastoma, osteosarcoma, medullary carcinoma of the thyroid, and extragonadal seminoma. Nitschke et al (11) reported one partial response (PR) in metastatic osteosarcoma, one PR in metastatic testicular embryonal carcinoma, and six responses among 25 patients with neuroblastoma. These six patients showed one complete response (CR), three PRs, and two subjective responses. Ochs et al (12) reported eight patients with advanced osteosarcoma treated with *cis*-platinum. They found four PRs and one CR.

Renal toxicity had previously restricted full evaluation of *cis*-platinum. In an attempt to ameliorate the acute focal necrosis of the distal convoluted tubules and collecting ducts (13), Merrin (14) advocated infusion of a dose of 1 mg/kg of *cis*-platinum over 6–8 hours together with 37.5 g of mannitol and 40 mg of furosemide in 200 ml of 5% dextrose in water and 0.3% sodium chloride. No renal toxicity was observed in 24 patients, six of whom had only one kidney. Using a similar program of aggressive hydration and diuresis, Hayes et al (15) escalated the dose to 3 mg/kg without encountering irreversible renal toxicity.

The Children's Cancer Study Group (CCSG) undertook the CCSG-071 study in order to determine the toxic and therapeutic effects of *cis*-platinum administered with aggressive hydration and diuresis in various childhood neoplasms. Seventeen patients with osteosarcoma (table 1) are evaluable. Three patients achieved stabilization of disease, two achieved a PR, and one achieved a CR. A discussion of this study and its results, together with plans for a phase III study with *cis*-platinum in osteosarcoma, follows.

[1]This study was conducted by the Children's Cancer Study Group and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare. The following institutions and investigators participated in the study: University of Southern California and Comprehensive Cancer Center, Los Angeles (Group Operations Office) (*D. Hammond, J. Weiner, R. Honour, H. Sather,* and *J. Anderson;* CA-13539); University of Michigan Medical Center, Ann Arbor (*R. Heyn;* CA-02971); Children's Hospital National Medical Center and George Washington University, Washington, DC (*S. Leikin;* CA-03888); Memorial Sloan-Kettering Cancer Center and Cornell University, New York, NY (*D. R. Miller;* CA-14557); Children's Hospital of Los Angeles and University of Southern California (*G. Higgins;* CA-02649); Babies Hospital and Columbia University, New York, NY (*J. Wolff;* CA-03526); Children's Hospital of Pittsburgh and University of Pittsburgh, Pa (*V. Albo;* CA-07439); Children's Hospital of Columbus and Ohio State University (*W. Newton;* CA-03750); Children's Orthopedic Hospital and Medical Center and University of Washington, Seattle (*R. Chard;* CA-10382); University of Wisconsin Hospitals, Madison (*N. Shahidi;* CA-05436); University of Minnesota Health Sciences Center, Minneapolis (*M. Nesbit* and *W. Krivit;* CA-07306); Children's Memorial Hospital and Northwestern University, Chicago, Ill (*G. Honig, P. Gaynon,* and *E. S. Baum;* CA-07431); University of Utah Medical Center, Salt Lake City (*E. Lahey;* CA-10198); Princess Margaret Hospital and University of Toronto, Ontario, Canada (*M. Sonley;* Ontario Cancer Treatment and Research Foundation grant 41-17); Strong Memorial Hospital and University of Rochester, NY (*M. Klemperer;* CA-11174); Children's Hospital and University of Louisville, Ky (*D. R. Kmetz*); University of British Columbia, Vancouver, Canada (*M. Teasdale*); Children's Hospital of Philadelphia and University of Pennsylvania (*A. Evans;* CA-11796); James Whitcomb Riley Hospital for Children and Indiana University, Indianapolis (*R. Baehner;* CA-13809); New Jersey College of Medicine and Dentistry, Newark (*L. Vitale;* CA-12637); Harbor General Hospital and University of California-Los Angeles, Torrance (*J. Finklestein* and *L. Greenberg;* CA-14560); University of California Medical Center, San Francisco (*A. Ablin;* CA-17829); Rainbow Babies and Children's Hospital and Case Western Reserve University, Cleveland, Ohio (*S. Gross;* CA-20320); Children's Hospital Medical Center and University of Cincinnati, Ohio (*B. Lampkin*); Vanderbilt University School of Medicine, Nashville, Tenn (*J. Lukens*); University of Oregon Health Sciences Center, Portland, Ore (*R. Neerhout*); University of Texas Health Science Center, San Antonio (*T. Williams*); and University of California Medical Center, Los Angeles (*S. Feig*).

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TABLE 1.—Patient characteristics

Patient No.	Sex/age	Primary tumor	Metastases of the— Lung	Bone	Previous chemotherapy*
1	M/16	Right femur	×	×	HD MTX, ADR, VCR
2	F/16	Right tibia	×		HD MTX, ADR
3	M/16	Left tibia	×		HD MTX, ADR, CPM/DTIC, L-ASP/pred
4	M/12	Left tibia	×		HD MTX, ADR
5	F/16	Right 3rd rib		×	HD MTX, ADR, VCR, bleo, AMD (T7)
6	M/14	Right femur	×		HD MTX, ADR, VCR
7	F/17	Right femur	×		CONPADRI-I, HD MTX, ADR
8	M/15	Left femur	×		HD MTX, ADR/CPM, VM-26, transfer factor
9	F/12	Right femur	×		HD MTX, ADR, VCR/AMD/CPM
10	F/15	Right femur	×		HD MTX, ADR
11	M/18	Left tibia	×		HD MTX, ADR, VM-26, CPM, VCR, VND
12	F/14	Left humerus	×		HD MTX, ADR, VND
13	M/16	Right tibia	×		HD MTX, ADR, CPM
14	F/16	Right femur		×	HD MTX, ADR, VCR
15	F/10	Left tibia	×		HD MTX, ADR
16	F/19	Right femur	×		VCR/CPM/AMD/ADR, HD MTX
17	M/16	Right femur	×		HD MTX, ADR

*HD MTX = high-dose methotrexate; ADR = Adriamycin; VCR = vincristine; CPM = cyclophosphamide; L-ASP = L-asparaginase; pred = prednisone; bleo = bleomycin; AMD = actinomycin D; CONPADRI-I = cyclophosphamide, vincristine, melphalan, and Adriamycin; VND = vindesine.

Patient Eligibility

Patients with recurrent or metastatic osteosarcoma resistant to Adriamycin (ADR) and high-dose methotrexate (MTX) were eligible for the study. At least one measurable lesion as noted by roentgenogram or isotope scan was required. As in other CCSG studies, informed consent was required in accordance with the Department of Health, Education, and Welfare informed-consent guidelines.

BUN, creatinine, uric acid, and 24-hour creatinine clearance had to be within normal limits. Serum electrolytes, including calcium and magnesium, were also required to be normal. The absolute neutrophil count had to be $> 1.5 \times 10^9$/liter and the platelet count had to be $> 75 \times 10^9$/liter.

Treatment Plan

Patients were hospitalized for treatment. *cis*-Platinum was administered at a dose of 3 mg/kg with aggressive hydration and diuresis every 3 weeks, with an optional escalation to 4.5 mg/kg after three courses if no response had been seen. Prior to infusion of the *cis*-platinum, serum creatinine, BUN, uric acid, calcium, magnesium, SGOT, bilirubin, blood cell count with platelets, 24-hour urine for creatinine clearance, serum electrolytes, weight, and blood pressure were obtained. One-half hour before beginning the *cis*-platinum infusion, an antiemetic was administered when appropriate and strict input and output were measured and reviewed hourly. The *cis*-platinum was protected from light and was administered at doses of 3 or 4.5 mg/kg in 1500 ml/m^2 of 5% dextrose in water and 0.45% sodium chloride with 250 mg/kg of mannitol and 1 mEq/kg of KCl over an 8-hour period.

After 4 hours, administration of any accumulated fluid deficit was begun as 5% dextrose in water and 0.45% sodium chloride with 20 mEq/liter of KCl and was continued over the next 4 hours. The antiemetic was repeated as needed. After 8 hours the infusion was terminated. Serum electrolytes, creatinine, weight, and blood pressure were rechecked. Iv fluid constituted to correct any electrolyte abnormalities was administered at a rate of 125 ml/m^2/hour for 16 hours. At the end of this time parenteral fluids were continued until oral fluids were tolerated.

If urine output fell to < 150 ml/m^2/hour during the *cis*-platinum infusion, 200 mg/kg of mannitol was given as a rapid infusion. If this did not induce a prompt diuresis, furosemide was administered at a dose of 0.5 mg/kg.

TABLE 2.—Response

Patient No.	No. of courses of *cis*-platinum at doses of— 3 mg/kg	4.5 mg/kg	Response*	Duration (wks)	No. of episodes of toxicity† Renal	Hearing	Calcium (mg/100 ml) ⩾ 8	Calcium (mg/100 ml) < 8	Magnesium (mEq/liter) ⩾ 1.2	Magnesium (mEq/liter) < 1.2	Myelosuppression Anemia	Thrombocytopenia	Neutropenia
1	3		PD				1	2	1		1§		
2	5		PD		1+				1	1			
3	9		PR	30	2+		3		4		1		2
4	1	5	PR	12									1
5		4	PD			2+					1§	2	1
6	3		PD			1+			1				
7	3	2	S/NP	10		1+			1				
8	2		PD										
9	3		PD								1		
10	2		PD				1‡				1	1	
11	1	3	S/NP	6	3+	1+		1					
12	3	1	PD				1		1		1		
13	3	4	PD		2+		2‡	2‡			1§		
14	6		PD				1‡		1	2			2
15	5		S/NP	8	2+	1+			1		1		
16	8		PD		2+		2	2	3	1	1		
17	1	1	CR	8+			1						

*PD = progressive disease; PR = partial response; S/NP = stabilization/no progression; CR = complete response.

†*Renal:* 1+ = transient BUN > 25 mg/100 ml, creatinine > 1.5 mg/100 ml, creatinine clearance 30% decrease; 2+ = same as 1+ but persisting beyond 3 wks; 3+ = BUN > 40 mg/100 ml or creatinine > 2.0 mg/100 ml. *Hearing:* 1+ = abnormal audiometry, no symptoms; 2+ = transient symptoms; 3+ = persistent symptoms. *Hypocalcemia* (< 9.0 mg/100 ml): mild = ⩾ 8.0 mg/100 ml; severe = ⩽ 7.9 mg/100 ml. *Hypomagnesemia* (< 1.5 mEq/liter): mild = ⩾ 1.2 mEq/liter; severe = < 1.2 mEq/liter. *Anemia* (hemoglobin < 9 g/100 ml). *Thrombocytopenia* (< 100.0 × 10^9 cells/liter). *Neutropenia* (< 1.5 × 10^9 cells/liter).

‡Baseline calcium < 9.0 mg/100 ml.

§Anemia complicated by hemoptysis in patient 1, by epistaxis in patient 5, and by serosanguineous pleural fluid in patient 13.

Tumor Response

A CR was defined as the disappearance of all measurable tumor. A PR was defined as a > 50% reduction in the sum of the products of two diameters of each measurable lesion with no lesion increasing in size and no new lesions. Stabilization/no progression (S/NP) was defined as the arrest of growth of all measurable lesions.

RESULTS

Seventeen patients were evaluable (nine males and eight females), ranging in age from 10 to 17 years with a median of 15½ years. Fourteen patients had disease confined to the lung, two patients had disease confined to the bone, and one patient had disease in both the lung and the bone. A total of 78 courses of *cis*-platinum were administered; each patient received between two and ten courses (median, four). Fifty-six courses were at a dose of 3 mg/kg and 20 courses were at a dose of 4.5 mg/kg. Seven of the 17 patients received at least one course at the higher dose.

Efficacy (table 2)

Ten of 17 patients experienced clear progression of disease. Four patients showed S/NP. The duration of stabilization was 4+, 6, 8+, and 10 weeks. One patient refused *cis*-platinum after two courses, but showed no enlargement of her lung metastases while receiving therapy. One patient experienced progressive disease while receiving three courses at 3 mg/kg, but showed arrested growth of her lung metastases for 10 weeks while receiving two courses at 4.5 mg/kg.

Three patients showed definite responses: two PRs and one CR. One patient with a PR showed shrinkage of his multiple pulmonary metastases that became apparent after two courses of *cis*-platinum. After four courses his chest roentgenogram was clear of metastatic disease, but nodules were seen on lung tomography. His response lasted a total of 7 months. The second patient with a PR received one course of *cis*-platinum at 3 mg/kg and one course at 4.5 mg/kg; his chest roentgenogram showed partial clearing of lung metastases. This effect had persisted for 5 months while receiving therapy when tumor

TABLE 3.—Onset of toxicity per course of *cis*-platinum

No. of courses	No. of trials	No. of episodes of toxicity*								
				Calcium (mg/100 ml)		Magnesium (mEq/liter)				
		Renal	Hearing	≤ 8	> 8	≤ 1.2	> 1.2	Anemia	Thrombo-cytopenia	Neutro-penia
1	17			4		1		2		
2	17		2	1	2	3	1	1	1	3
3	14	2	1	4	2	5		1	1	1
4	11	1	1		1	1	2	2	1	
5	8	1	1	1	1	1		1		2
6	5	1			2	1	2	1		
7	3	1		1		2				
8	2			1						
9	1							1		

*See footnotes in table 2 for definitions.

recurred. The patient who achieved a CR also had only lung metastases. He received one course of *cis*-platinum at 3 mg/kg and showed definite roentgenographic evidence of improvement at the time of his second course. He then received one course at 4.5 mg/kg and achieved disappearance of all lung metastases on roentgenogram. He refused further therapy because of the pain of venipuncture, but still had a CR 2 months after the cessation of therapy at the time of his last evaluation.

Toxicity (table 3)

Nausea and vomiting were reported in 22 courses: 12 cases at 3 mg/kg and ten cases at 4.5 mg/kg. At the lower dose, no patient was described to have severe nausea and vomiting, but nausea and vomiting were described as moderate in five patients and mild in seven patients. At 4.5 mg/kg, nausea and vomiting were described as severe in three patients, moderate in four patients, and mild in three patients.

Some degree of renal impairment was seen in six patients. Patient 2 maintained a normal serum creatinine throughout therapy. However, the creatinine clearance declined gradually from 107 ml/minute prior to *cis*-platinum to 66 ml/minute after three courses of the drug. At the same time the BUN level rose to 24 mg/100 ml from a pretreatment level of 10 mg/100 ml. After four courses of *cis*-platinum the creatinine clearance returned to 103 ml/minute and the BUN level fell to 9 mg/100 ml. After five courses the BUN level was 10 mg/100 ml and the creatinine was 1.0 mg/100 ml, with a pretherapy level of 0.6 mg/100 ml. Patient 3 had a creatinine clearance of 138 ml/minute after one course of *cis*-platinum; after six courses it was 75 ml/minute and after eight courses it was 45 ml/minute. Coincidentally, the serum creatinine rose from 0.7 mg/100 ml before treatment to 1.3 mg/100 ml after seven courses and 1.7 mg/100 ml after nine courses. Patient 11 had a rise in the serum creatinine of from 0.5 mg/100 ml before treatment to 1.9 mg/100 ml after three courses of *cis*-platinum. After a fourth course the BUN rose to 91 mg/100 ml. The BUN had not returned to normal at the time of death 10 weeks later. The patient was not oliguric. In patient 13, *cis*-platinum was stopped after seven doses because of elevation of the serum creatinine to 1.9 mg/100 ml. The creatinine remained elevated for at least 6 weeks. Patient 15 had an elevated BUN of 32 mg/100 ml after four courses of *cis*-platinum. The creatinine clearance had declined from 170 ml/minute before treatment to 87 ml/minute after one course and 71 ml/minute after four courses. The serum creatinine rose from 0.4 to 0.9 mg/100 ml. Patient 16 had a rise in the BUN to 30 mg/100 ml after the fifth course of *cis*-platinum. The BUN rose to 38 mg/100 ml and the serum creatinine rose from 0.8 to 1.4 mg/100 ml after the sixth course. She tolerated two more courses of *cis*-platinum before the disease showed definite evidence of progression.

Five of six patients in whom audiometry was performed had a high-frequency hearing loss of > 10 decibels. One of the five patients (No. 5) complained of transient tinnitus and difficulty in hearing speech.

Hypocalcemia was defined as a calcium value of < 9.0 mg/100 ml. Mild hypocalcemia was defined as a value of > 8.0 mg/100 ml and severe hypocalcemia was defined as a value of ≤ 8.0 mg/100 ml. Nineteen episodes of hypocalcemia were seen. Three patients who were hypocalcemic prior to receiving any *cis*-platinum (No. 10, 8.7 mg/100 ml; No. 13, 7.8 mg/100 ml; and No. 14, 7.6 mg/100 ml) experienced episodes of hypocalcemia. Two patterns were seen. The most severe hypocalcemia occurred within 24 hours after the *cis*-platinum infusion, with the lowest calcium value being 6.2 mg/100 ml. The second pattern was more chronic, with a gradual decline from course to course. No patient was symptomatic.

The lower limit of normal for magnesium was defined as 1.5 mEq/liter. Severe hypomagnesemia was defined

as a magnesium level of < 1.2 mEq/liter. Nineteen episodes of hypomagnesemia were noted. Again, these were of two kinds: acute (in the 24 hours following the *cis*-platinum infusion) and chronic (a gradual decline in the serum magnesium from course to course). The lowest magnesium reported was 0.8 mEq/liter. No patient was symptomatic from hypomagnesemia.

Nine patients developed anemia (hemoglobin < 9 g/100 ml) while being treated with *cis*-platinum. In three patients the etiology is uncertain. Patient 1 had hemoptysis. Patient 5 had recurrent epistaxis. Patient 13 had recurrent pleural effusions. More than 12 liters of serosanguineous pleural fluid were removed during the 5 months he was in the study.

The lowest platelet count reported after *cis*-platinum treatment was 23.0×10^9/liter for patient 5 after three courses. She received a fourth course with that platelet count and no further decline was seen. A second patient, No. 10, had a platelet count of 60.0×10^9/liter after two courses of *cis*-platinum. No other patient had a platelet count of $< 100.0 \times 10^9$/liter.

Four patients had six episodes of neutropenia (absolute neutrophil counts [ANC] of $< 1.5 \times 10^9$/liter). The lowest count was 0.28×10^9/liter 3 weeks following a second course of *cis*-platinum. A third course was given despite the neutropenia and 3 weeks later the patient (No. 14) had an ANC of 1.90×10^9/liter. No other ANC was $< 0.9 \times 10^9$/liter.

Patient 3 experienced transient erythema of the upper part of his body. The reaction was promptly controlled with diphenhydramine, but recurred during the next exposure to *cis*-platinum.

DISCUSSION

Nausea and vomiting were common but not as common or significant as might be expected from previous reports (1). Hayes et al (15) expressed a suspicion that mannitol-induced diuresis may lessen the emesis induced by *cis*-platinum.

Renal toxicity was significant in six of 17 patients, although none of the patients were oliguric or required dialysis. It occurred in four of ten patients receiving *cis*-platinum at a dose of 3 mg/kg and in two of seven patients at 4.5 mg/kg. However, patients with renal impairment received a median of six courses of *cis*-platinum (four, five, five, seven, eight, and nine courses). Those without renal impairment received a median of three courses (two, two, two, three, three, three, four, four, five, six, and six courses). No patient had received an aminoglycoside. Unfortunately, interval evaluation of renal function is not available for patients no longer in the study. Dentino et al (16) reported a regular and persistent decrease in the creatinine clearance in patients with testicular carcinoma receiving *cis*-platinum. These patients received 5-day courses of *cis*-platinum at 20 mg/m^2/day every 3 weeks without aggressive hydration or diuresis; no recovery of renal function was seen. The nephrotoxicity of *cis*-platinum appeared enhanced by administration of an aminoglycoside antibiotic 7–10 days following *cis*-platinum. Overall, a 40% decrease in the glomerular filtration rate was seen. At 12, 18, and 24 months the BUN was only slightly above the normal range. No correlation was found between the total dose of *cis*-platinum received and the decrease in creatinine clearance.

Hearing loss in five of six patients tested is disturbing. Somewhat reassuring is the fact that the problem was clinically evident in only one of the 17 patients.

Hypocalcemia and hypomagnesemia, although common, were asymptomatic. Since the steepest drops were seen in the 24-hour period following *cis*-platinum infusion, the 8- and 24-hour serum levels must be obtained as per the treatment protocol. Oral supplementation may ameliorate the gradual decline in these values seen from course to course. The acute decrease in these values may be secondary to dilution from the high volume of iv fluids administered.

As expected (1), bone marrow toxicity was mild. This is a significant advantage for the use of *cis*-platinum in combination chemotherapy.

In this study all patients had had prior exposure to high-dose MTX with citrovorum factor (CF) rescue and ADR. Three objective responses (one PR and two CRs) were seen among 16 patients. Response was evident after two courses.

This study corroborates the observation by Ochs et al (12) that *cis*-platinum is an active agent in osteosarcoma. Although no response was seen in this study in any of three patients with bony lesions, Ochs et al (12) reported one PR in a patient with bony disease. Data for single-agent trials of high-dose MTX and CF or ADR are not dissimilar (table 4). In 1972, Jaffe (17) obtained two CRs and two PRs in ten patients with osteosarcoma with high-dose MTX and CF. In 1974, Pratt et al (18) reported no responses among four patients. With ADR as a single agent, Gottlieb (19) reported an objective response in 18 of 81 patients in 1975. In 1978, Benjamin et al (20), reporting on the experience of the M. D. Anderson Hospital and Tumor Institute (Houston, Tex), found three PRs among 11 patients. The results with *cis*-platinum reported by Ochs et al (12) and in this study seem of similar magnitude.

These data do not demonstrate any optimal dose or schedule for *cis*-platinum. Eight of ten patients treated solely at 3 mg/kg had definite disease progression. One PR was noted. Of seven patients who received at least one course at 4.5 mg/kg, three had definite disease progression. One CR and one PR were obtained. This experience with the 4.5-mg/kg dose is inadequate to evaluate efficacy. Toxicity seems not vastly different. In testicular

TABLE 4.—Single-agent trials in osteosarcoma

Drug	Ref No.	No. of patients	No. of responses		Total No. of responses
			CR	PR	
High-dose MTX + CF	17	10	2	2	4
High-dose MTX + CF	18	4	0	0	0
ADR	19	81	—	—	18
ADR	20	11	0	3	3
cis-Platinum	12	8	1	4	5
cis-Platinum	Current study	16	1	2	3

carcinomas, Hayes et al (15) noted responses at doses of 3 mg/kg in six patients who were unresponsive at doses of 1 mg/kg. Wiltshaw and Kroner (5), on the other hand, found no difference in response at 30 mg/m^2 given in one dose every 2–3 weeks and at 30 mg/m^2 given for 3 days every 3–4 weeks in patients with ovarian carcinoma.

Experience with *cis*-platinum as adjuvant therapy is being obtained. Ochs et al (12) reported a single patient with osteosarcoma of the mandible who experienced a local recurrence after subtotal resection and adjuvant ADR chemotherapy. She subsequently had further resection and received adjuvant *cis*-platinum for 4 months; she remains disease-free at > 37 months. An additional patient was treated at Children's Memorial Hospital (Chicago, Ill). She had osteosarcoma of the pelvis and had had a hindquarter amputation and received adjuvant chemotherapy with ADR. After 4 months she developed a thoracic (T8) vertebral metastasis. She had a biopsy followed by partial excision, and then received radiation therapy to 5000 rads and *cis*-platinum at 3 mg/kg as adjuvant chemotherapy (11 courses). She remains disease-free for > 20 months. Ettinger et al (21) reported his experience with eight consecutive patients with localized osteosarcoma treated with surgery and adjuvant *cis*-platinum and ADR. Eight of eight patients are disease-free with followup at 3–21 months. The CCSG is planning a phase III study in recurrent metastatic osteosarcoma comparing weekly high-dose MTX and CF with alternating weekly courses of high-dose MTX and CF and *cis*-platinum. Altered MTX clearance secondary to *cis*-platinum-induced renal damage is a real concern. Further studies appear indicated.

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Germ Cell Tumors of the Testis

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SUMMARY

The histologic patterns of the various germ cell tumors of the testis are reviewed and these tumors have been defined and classified according to the World Health Organization criteria. The importance of obtaining adequate sections of the primary and metastatic tumors is stressed and more extensive use of the World Health Organization classification of testicular tumors is encouraged so that meaningful comparisons may be made between different centers treating these patients.

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There has been much confusion as to the best manner in which testicular tumors should be classified (1). This is due to the great potential for germ cells to differentiate in a multiplicity of directions. In view of the histologic diversity of these tumors, I shall first describe the individual histologic patterns they may take and, after this, attempt to put them together into a meaningful classification. Figure 1 illustrates the various normal structures to which testicular tumors would be considered analogous.

SEMINOMA

Seminoma is a tumor with an age incidence peak of between 35 and 39 years, consisting of fairly uniform cells typically with clear cytoplasm (glycogen-rich) and resembling primitive germ cells. The sheets of cells in a typical seminoma are divided by delicate fibrous septae. They often contain numerous mature small lymphocytes. The cell walls are clearly seen, and are useful in distinguishing seminoma from embryonal carcinoma (2). The nuclei are round, with prominent nucleoli, and have occasional mitotic figures. The tumor is designated "anaplastic" if there are three or more mitoses per high-power field and/or if there is considerable nuclear variation (3). This latter type constitutes approximately 10% of seminomas (4). Lymphocytes are less prominent in this group than in the usual seminoma (4). Approximately 30% of seminomas will show a granulomatous reaction in their stroma with or without foreign body or Langhans' giant cells (5). This granulomatous reaction may overrun the tumor to a marked degree, leaving only a few recognizable tumor cells. Syncytial giant cells may be found in these tumors and these may resemble syncytiotrophoblast (6). These cells stain positively for human chorionic gonadotropin (HCG) using immunoperoxidase techniques (7). They may be found in approximately 6% of patients (5). These cells are thought to indicate a somewhat worse prognosis than for seminomas without them (6). The degree of lymphocytic infiltration has been correlated with the prognosis of seminomas: the more lymphocytes, the better the prognosis (5). It is thought that anaplastic seminomas are the tumors responsible for a high proportion of the more aggressively metastasizing seminomas (4,8). Seminomas may undergo spontaneous regression, with the tumor being replaced partially or even totally by dense hyalinized scar tissue. These scars may be very small and require a careful search on the part of the pathologist to be found. It is important to search for the presence of such a scar before one accepts a retroperitoneal seminoma (or other germ cell tumors) as being primary in the retroperitoneum (9).

SPERMATOCYTIC SEMINOMA

Spermatocytic seminoma is a distinctive tumor first described by Masson (10), in which the cells vary a great deal in size from lymphocyte-like to giant cells about 100 μm in diameter. The bulk of the tumor, however, is com-

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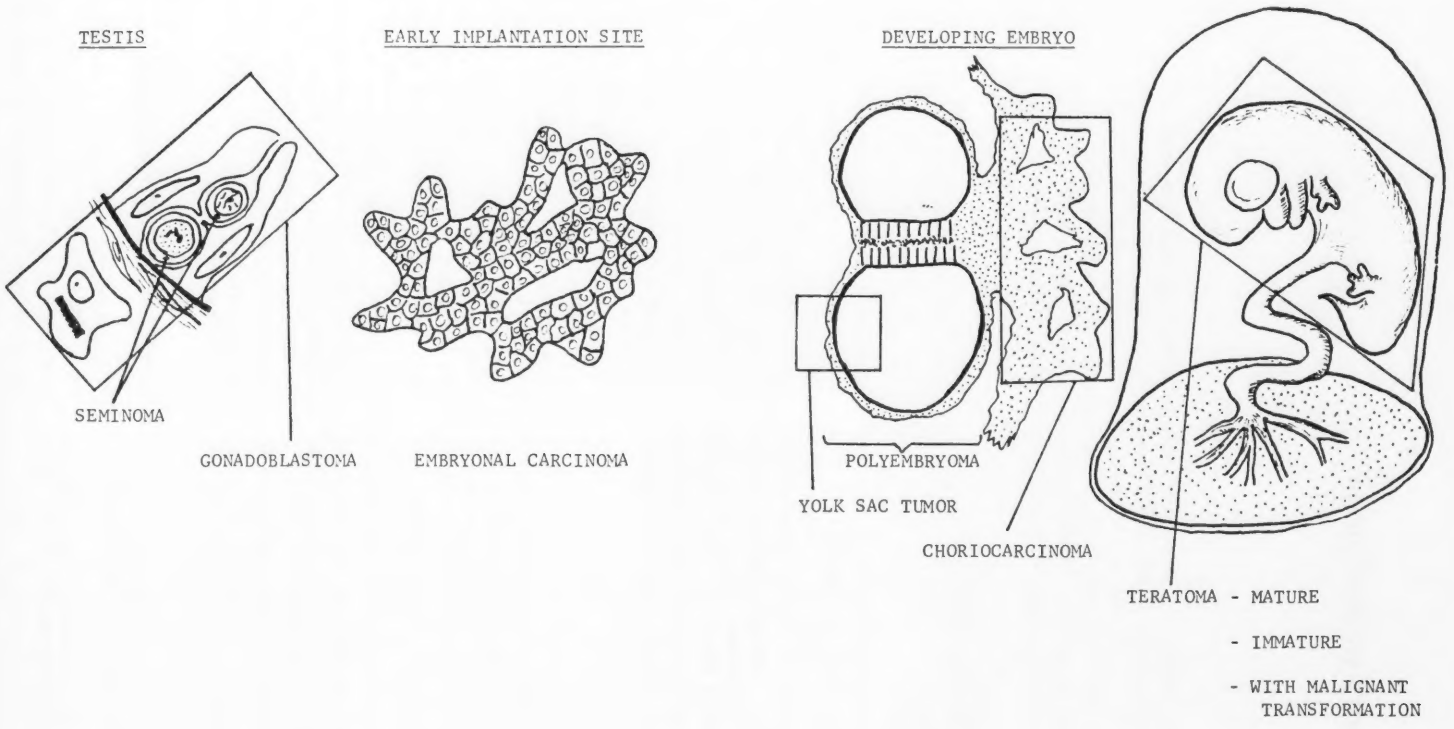

FIGURE 1.—Testicular tumors.

posed of cells of intermediate size. These uncommon tumors occur in an older age group than the usual seminoma, the age peaking between 45 and 49 years. They account for most seminomas found in patients after the age of 70 and constitute 3.7% of seminomas (5). Grossly, they tend to be larger than the usual seminoma. They may be cystic, are more frequently multiple, and are more frequently bilateral, with a bilaterality rate of approximately 6% (11) as opposed to 1% in the usual seminoma (4). Microscopically, they have the characteristics described above and the nuclei of the large cells show a typical spireme-type pattern resembling the pattern seen in spermatocytes of the normal testis. These tumors do not have a lymphocytic infiltrate or a granulomatous reaction and they have an extremely good prognosis. Very few examples in the literature have demonstrated metastatic capabilities (11).

GONADOBLASTOMA

Gonadoblastoma is a tumor composed of two principal cell types: large germ cells similar to those of seminoma and small cells resembling immature Sertoli and/or granulosa cells. Elements resembling Leydig cells may also be present. This is a very uncommon tumor and it will not be discussed in detail. It occurs almost invariably in abnormal (often dysgenetic) gonads and the patients frequently exhibit chromosomal mosaicism, almost always having a demonstrable Y chromosome present (12). The tumor appears to recapitulate the embryonic development of the gonad, including both the germ cell and stromal elements (4). The germ and Sertoli cells are surrounded by a basement membrane and globules of basement membrane material are found between the cells (13). Occasional laminated calcospherules are seen and these have been shown by Garvin et al (13) to contain nuclear material from degenerate germ cells in their centers. About 50% of these tumors are associated with the development of a germinoma, but they may also develop other forms of malignant germ cell tumor capable of metastasis (4,12).

EMBRYONAL CARCINOMA

Embryonal carcinoma is a tumor composed of cells of primitive epithelial appearance, often with clear cytoplasm, growing in a variety of patterns. These tumors could correspond to the early implantation site before the embryo shows any evidence of differentiation at all, and may be the neoplastic progenitor of the other germ cell tumors (14). They occur in a younger age group than seminoma, the age peaking between 25 and 29 years. Grossly, they tend to be necrotic and hemorrhagic, and microscopically they usually show more organization than seminomas, with gland-like spaces, papillary structures, and trabeculae. There may, however, be areas of solid tumor difficult to differentiate from seminoma. The cells are somewhat larger than the cells of seminoma with prominent nucleoli and dense nuclear membranes. They are closely adherent to one another and the cell borders are indistinct. The cytoplasm is somewhat more basophilic than in seminoma although it usually contains glycogen as well. Immunoperoxidase techniques have demonstrated alpha-fetoprotein in the tumor cells (7,14). Mitotic figures are frequent and the tumor has a very anaplastic appearance overall. It frequently has a somewhat immature myxomatous-appearing stroma and this should not be considered teratomatous in nature (3) since it is part of the embryonal carcinoma. Occasionally, these tumors may contain syncytial giant cells resembling syncytiotrophoblast, and it is important not to misdiagnose these cells as choriocarcinoma. Choriocarcinoma should only be diagnosed when the strict criteria for such a tumor have been met. They do, however, show the presence of HCG on immunoperoxidase staining (7,14). Very occasionally, there may be a granulomatous response, as shown by Tiltman (15).

POLYEMBRYOMA

Polyembryoma is a very uncommon tumor. Histologically, it shows evidence of differentiation towards the embryonic disc, with yolk sac and/or amniotic cavities being present on either side of the disc (16) (ie, the embryoid body), and this forms the predominant element. Immature loose stroma separates the bodies from one another and trophoblastic cells may be found in the intervening tissues. This tumor is thought to behave in a manner similar to the embryonal carcinoma and is usually classified with that tumor.

YOLK SAC TUMOR

The yolk sac tumor is one of the most common germ cell tumors of childhood and infancy (17). Yolk sac components, however, are also common in adult germ cell tumors. Talerman (18) found these elements in 38% of his patients with adult germ cell tumors of the testis. They are almost invariably associated with other germ cell elements in the adult. Yolk sac elements produce alpha-fetoprotein which may be demonstrated using immunoperoxidase techniques (7,14). There is a much better prognosis in children than in adults. If the child is < 2 years old and particularly if the history is short, the prognosis is good (17,19). In the adult, however, the presence of this pattern in a germ cell tumor is an adverse prognostic feature (18). This tumor corresponds to the embryonic yolk sac and histologically shows a loose pattern of growth with many clear spaces. The spaces are separated by thin strands of malignant cells. These cells

are occasionally arranged in the form of a Schiller-Duval body which consists of a central vessel surrounded by a mantle of cuboidal to columnar cells, and lies within a space. The tumor cells may blend with vascular channels, indicating an angiogenic potentiality for this tumor. Microscopically it may have a microvacuolar, reticular, tubular, or papillary pattern. The clear spaces are filled with acid mucopolysaccharides. There may be solid areas within the tumor, in which case it becomes difficult to differentiate this from embryonal carcinoma. Hyaline droplets are frequently seen within the cytoplasm of tumor cells and lying free between the tumor cells. These droplets have been shown to contain both alpha-fetoprotein and alpha-1-antitrypsin (20). They stain strongly with periodic acid-Schiff reagent, and are resistant to diastase digestion. They may be analogous to similar droplets seen in the liver in alpha-1-antitrypsin deficiency (2).

CHORIOCARCINOMA

Choriocarcinoma is a highly malignant tumor with a markedly hemorrhagic and necrotic gross appearance; careful sampling of these hemorrhagic areas is required in order to demonstrate recognizable, viable tumor. Microscopically there is extensive hemorrhage and necrosis. To diagnosis choriocarcinoma, not only must both syncytiotrophoblast and cytotrophoblast be identified, but they must be arranged in the typical plexiform pattern, with the syncytiotrophoblast capping the masses of cytotrophoblast and projecting into a space (usually containing rbcs) or forming the advancing front of the tumor (21). The Testicular Tumor Panel and Registry in Great Britain also requires that these tumors have a papillary configuration (22). It is important to be rigid in applying these criteria in view of the serious prognostic implications of this diagnosis. The tumor spreads predominantly via the blood stream, but also to retroperitoneal lymph nodes in a high percentage of patients (21).

TERATOMA

Teratoma is a germ cell tumor in which the cells are embryonic in nature, as opposed to extraembryonic as in the placenta and yolk sac; they must also demonstrate varying degrees of differentiation beyond the embryonic disc stage of development. Histologically the tissues may be immature, such as neuroblastoma-like areas or primitive neural tubes. Such areas do not necessarily denote malignancy even in the presence of necrosis. They may be mature, resembling adult tissues histologically, and may rarely undergo malignant change, forming, for example, squamous carcinoma, adenocarcinoma, or sarcoma. This is to be distinguished from the combined tumors (*see* below) of teratoma with embryonal carcinoma (previously designated "teratocarcinoma"). Teratomas occurring in patients < 12 years of age have a benign clinical course (17), but in patients > 12 years of age they are certainly capable of metastasizing, even if the pathologist finds only mature elements present in the primary tumor. Twenty-nine percent of such patients develop metastatic disease in the retroperitoneal lymph nodes during the course of the illness (21). Grossly these tumors appear solid with small cystic areas, and foci of calcification may be found. Histologically the possible variety of tissues is great, but the most common tissues seen include glandular structures, cartilage, squamous epithelium, and mature glial tissue.

DERMOID CYST

Dermoid cysts are seen frequently in the ovary, but are very uncommon in the testis and should be classified under mature teratomas. They have both epidermal and skin adnexal structures in the wall, together with teeth and other bony and cartilaginous tissues. Mostofi and Price (4) have not seen a case of pure dermoid cyst which metastasized.

EPIDERMOID CYST

Epidermoid cysts, on the other hand, consist of stratified squamous epithelium with keratinization, but without skin adnexal structures (4). They occur in the region of the rete testis and are not considered to be part of the spectrum of teratomas. When confronted with such an appearance pathologists must make certain that there are no adjacent scars and no other embryonal or teratomatous elements present in the adjacent tissue before designating such a lesion an epidermoid cyst.

TUMORS OF MORE THAN ONE HISTOLOGIC TYPE

Tumors of more than one histologic type are frequent among germ cell tumors of the testis, accounting for almost 40% of cases (19), and it is for this reason that pathologists should obtain extensive sections of primary testicular tumors in order to demonstrate all the relevant histologic elements present in the tumor. Any combination of the different histologic varieties described above may be found in this group of tumors. The most frequent form of combined tumor is embryonal carcinoma together with teratoma, either mature or immature. This was previously called "teratocarcinoma." The elements present, together with their proportions, should be stated in the pathology report. Tumors containing choriocarcinomatous elements (by rigid criteria) are categorized separately because of the serious prognosis implied by this diagnosis. Pure choriocarcinoma has the worst prognosis,

but if choriocarcinoma is combined with other teratomatous elements, the prognosis is slightly better (21). Seminoma is often combined with the other tumor types. The prognosis will generally depend on the nonseminomatous component of the lesion.

CLASSIFICATION OF GERM CELL TUMORS

The World Health Organization (WHO) published a classification of testicular tumors in 1977 in the hope that pathologists would attain uniformity in the reporting of these various tumor types so that results of therapy could be compared (3). It is the hope of Mostofi et al that this classification will be put into general use. The WHO classification of testicular tumors is as follows:

I. Germ cell tumors
II. Sex cord stromal tumors
III. Tumors and tumor-like lesions containing both germ cell and sex cord/stromal elements
IV. Miscellaneous (A. carcinoid)
V. Lymphoid and hematopoietic tumors
VI. Secondary tumors
VII. Tumors of collecting ducts, rete, epididymis, spermatic cord, capsule, supporting structures, and appendices (E. melanotic neuroectodermal tumor)
VIII. Unclassified tumors
IX. Tumor-like lesions (includes epidermoid cysts)

Germ cell tumors form > 90% of the total group in this classification and form the subject of this discussion. Tumors containing both germ cell and sex cord/stromal elements (group III of the WHO classification) have also been included for the sake of completeness. It should be noted that the carcinoid tumors, when occurring alone (ie, without other teratomatous elements), are classified as primary testicular carcinoid tumors under group IV (miscellaneous). The melanotic neuroectodermal tumors are classified in group VII because these occur largely in the paratesticular tissues and not often within the testis itself. The epidermoid cysts have been mentioned above and are included among tumor-like lesions. The subdivisions of germ cell tumors in the WHO classification are as follows:

I. Germ cell tumors
  A. Tumors of 1 histologic type
    1. Seminoma (including anaplastic seminoma)
    2. Spermatocytic seminoma
    3. Embryonal carcinoma
    4. Yolk sac tumor (embryonal carcinoma, infantile type, endodermal sinus tumor)
    5. Polyembryoma
    6. Choriocarcinoma
    7. Teratoma
      a. Mature
      b. Immature
      c. With malignant transformation
  B. Tumors of > 1 histologic type
    1. Embryonal carcinoma and teratoma
    2. Choriocarcinoma and any other types; specify type (this combination has a grave prognosis: 20% 5-year survival)
    3. Other combinations; specify types

They are divided broadly into tumors of one histologic type and tumors of more than one histologic type. The single-tumor types discussed earlier are placed in the first group when they occur alone, and combinations of these are placed in the second group. Teratomas are designated "immature" when immature (but not malignant) elements are present, and "mature" when all the tissues are histologically mature. If any of these tissues have undergone malignant change, eg, to adenocarcinoma or spindle cell sarcoma, the tumor is designated "teratoma with malignant transformation." This must be distinguished from the combined tumor of teratoma and embryonal carcinoma (previously designated "teratocarcinoma"). The latter designation has been deleted because it is preferable to designate the specific tumor types present and their proportions. Choriocarcinoma, if it conforms to strict criteria, is classified as a separate group no matter what tumor type accompanies it. All other combinations are grouped in group 3 (other combinations). Table 1 is modified from the reports of two large series of patients, namely, the Testicular Tumor Panel in 1976 (22) and Nefsger and Mostofi in 1972 (23). This illustrates the relative incidence of the various types of tumors which have been broken down according to the WHO classification. The common tumors seen in this group include seminoma, embryonal carcinoma, and combinations of embryonal carcinoma and teratoma, as well as the other combinations. Choriocarcinoma in its pure form is a very uncommon tumor (0.3% of testicular tumors).

The germ cell tumors of the testis have characteristic patterns of spread. Seminomas spread predominantly via lymphatic channels to the retroperitoneal lymph nodes. If these tumors spread beyond the testis into the epididymis, they gain access to lymphatics draining to the deep iliac nodes (2) as well as to the high retroperitoneal lymph nodes (the primary drainage of the testis). A small proportion of seminomas will metastasize via the blood stream to the lungs and other distant organs (21). Embryonal carcinoma alone and combined with teratoma spread via both the lymphatics and the blood stream, and choriocarcinoma is notorious for blood vessel invasion and spread of the tumor via the blood stream (21). It also spreads to the retroperitoneal nodes in the majority of

TABLE 1.—Incidence of various tumor types from 2 reports according to the WHO classification

	Incidence of tumor types	
	Testicular Tumor Panel* (1976)	Nefsger and Mostofi† (1972)
Tumors of 1 histologic type		
Seminoma	38.0	28.2
Anaplastic seminoma	—	3.4
Spermatocytic seminoma	1.5	3.1
Embryonal carcinoma	11.6	13.3
Yolk sac tumor	1.9	—
Polyembryoma	—	—
Choriocarcinoma	—	0.3
Teratoma		
Mature	—	—
Immature	1.5	6.5
Malignant	—	—
Tumors of > 1 histologic type		
Embryonal carcinoma and teratoma	17.4	21.8
Choriocarcinoma and any other type	1.2	2.4
Other combinations	13.5	12.7
Totals	86.6	91.7
Non-germ cell tumors	13.4	8.3
Totals	100.0	100.0

**From* ref 22.
†*From* ref 23.

patients (21). An important point to note regarding metastatic lesions from these tumors is that the histology of the metastasis may appear different from the primary tumor (21). An embryonal carcinoma primary in the testis may give rise to a metastasis in the retroperitoneum containing teratomatous or choriocarcinomatous elements. This is uncommon, occurring in 8% and 5% of cases respectively. This may represent inadequate sampling of the primary lesion but it is conceivable that these cells may mature into either teratomatous or choriocarcinomatous elements in their metastatic sites. The influence of therapy in this regard is of interest and it is possible that chemotherapy and/or radiation therapy may play a significant role in this transformation (24). Primary testicular seminoma may be associated with embryonal carcinoma, teratoma, or choriocarcinoma in its metastases, although fortunately, this is extremely uncommon (5). Choriocarcinoma will spread as choriocarcinoma (21). It is well-known that mature teratomas may give rise to metastatic disease in the retroperitoneum. This may be because small foci of embryonal carcinoma are still present within the tumor but are not recognized, and these then result in metastatic deposits which mature (24); however, we have seen at least one patient in whom mature teratomatous elements have been found within the venous channels draining the testis. It is conceivable that these mature elements may be capable of seeding distant sites as well. Experimental evidence does not support this, however (1).

TABLE 2.—Comparison of the WHO and the British classifications

WHO (1977)	Testicular Tumor Panel (1975)
Teratoma, mature	Teratoma, differentiated
Teratoma, immature	
Teratoma with malignant transformation	Malignant teratoma, intermediate
Embryonal carcinoma and teratoma	
Choriocarcinoma and any other type (specify type)	Malignant teratoma, trophoblastic
Embryonal carcinoma	Malignant teratoma, undifferentiated
Other combinations (specify types)	Combined tumor (seminoma and teratoma)

Table 2 shows a comparison between the British and the WHO classifications, and it will be noted that, although there are conceptual differences, the substantive differences between these two classifications are not very great.

Testicular tumors represent a challenge both to the pathologist and to the therapist involved in the management of these patients. It is important to stress the necessity for obtaining adequate numbers of sections from both the primary and metastatic tumors when they are received in the laboratory. This will minimize the difficulties in classifying the tumor correctly and in predicting the likely behavior of the lesion. It would also be of great benefit if pathologists would adopt a uniform classification so that adequate comparisons can be made between different centers treating these patients in this era of rapidly advancing chemotherapy.

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Staging Procedures and the Role of Surgery in Testicular Cancer

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SUMMARY

The conventional staging system for testicular cancer is inadequate in discriminating between varying degrees of local, nodal, and pulmonary disease. This staging system which uses conventional tests including lymphangiogram, iv pyelogram, and inferior venacavogram has been demonstrated to have a 35%–53% error rate in distinguishing between stages I and II cancer based upon retroperitoneal node dissection. A surgicopathologic staging system has been proposed which improves upon the conventional staging system for testicular cancer. This clinical and surgicopathologic staging system has been proposed based on determination of serum alphafetoprotein and human chorionic gonadotropin before and/or after orchiectomy and lymphadenectomy with a low staging error. The conventional surgical management of testicular cancer and the current status of the National Cancer Institute prospective randomized clinical trial assessing the role of intensive chemotherapy with or without cytoreductive surgery in poor-risk bulky stage III testicular cancer are discussed.

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As in the staging systems used for other tumors, the staging of testicular tumors must accurately reflect the extent of the patient's disease. However, it should equally reflect the natural history of disease progression, allowing the physician to make an accurate assessment of the disease, select the appropriate treatment, and gain some insight as to the patient's prognosis. The conventional staging of testicular tumors was described by Boden and Gibb (1). Although it does allow prognostication and reflects tumor extent to some degree, it does not provide a precise evaluation of the dissemination and natural history of the disease. The major problems with this staging method are as follows. First, there is no means to discriminate between varying degrees of local spread within the category of stage I disease which results in lumping patients with localized invasive and noninvasive diseases together. Second, the stage II designation does not adequately reflect the variable degree of regional lymphatic involvement and thus combines patients with minimal disease (potentially surgically curable) with patients who have massive surgically noncurable disease. Third, the stage III category lacks the provision for describing minimal or maximal disease.

Based upon these observations, a staging classification is proposed which potentially will increase our ability to determine extent of disease, will assist in determining appropriate therapy, and will improve our prognostic skills (2) (fig 1). This staging method for testicular cancer takes into consideration the factors which are essential in the therapy for and prognosis of this disease.

Stage I: Local spread
- P1 – Confined to testis
- P2 – Involves testicular adnexa
- P3 – Involves scrotal wall

Stage II: Confined to retroperitoneal lymphatics
- N1 – Microscopic
- N2 – Gross involvement without capsular invasion
- N3 – Gross involvement with capsular invasion
- N4 – Massive involvement of retroperitoneal structures

Stage III: Beyond retroperitoneum
- M1 – Solitary metastasis
- M2 – Multiple metastases

(P = Primary, N = Node, M = Distant metastasis)

FIGURE 1.—Surgical pathologic staging for testicular cancer.

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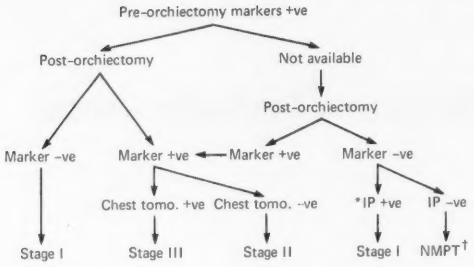
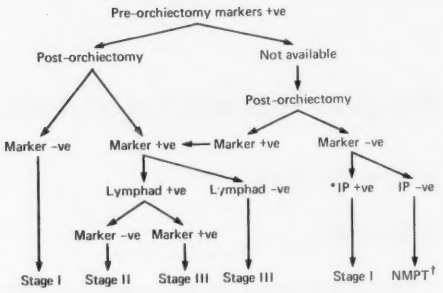


*IP = Immunoperoxidase
†NMPT = Non marker producing tumor

FIGURE 2.—Clinical staging for testicular cancer based on tumor markers (HCG and AFP).



*Immunoperoxidase
†Non marker producing tumor

FIGURE 3.—Surgical pathologic staging for testicular cancer based on histology and tumor markers (AFP and HCG).

Using the proven efficacy of the tumor-derived markers alphafetoprotein (AFP) and human chorionic gonadotropin (HCG), we have also proposed a clinical and a surgicopathologic staging based on preorchiectomy or postorchiectomy and postlymphadenectomy determination of serum HCG and AFP.

Clinical Staging for Testicular Cancer Based on Tumor Markers

Determination of serum HCG and AFP appears to allow staging of a patient with testicular cancer more accurately than other clinical tests. Using these markers we have decreased the staging error rate from 53% (3) reported for embryonal carcinoma to 14% (4). In interpreting these markers care must be taken to consider their biologic half-lives (5 days for AFP and 18–24 hours for HCG). Also, it is our experience that 10% of nonseminomatous and 92.5% of seminomatous testicular tumors do not produce markers and we have designated them as non-marker-producing tumors. Using the markers and considering the above provisions we have proposed a clinical staging system based on the serum marker determinations (fig 2).

Surgicopathologic Staging Based on Tumor Markers

We have also proposed a staging system (fig 3) based on surgical and pathologic material and determination of serum HCG and AFP. Although preorchiectomy determination of the serum markers is not always available, this should not greatly disturb this staging system since the original specimen from orchiectomy is usually available and immunochemical techniques such as immunoperoxidase can determine the presence of cellular markers when serum is not available.

Parameters for Staging

In addition to the conventional laboratory and radiologic procedures used in clinically staging patients with testicular tumors, we have investigated the efficacy of chest tomography, computerized axial tomography of the retroperitoneum, tumor markers, inferior venacavogram, iv pyelogram, and lymphangiogram. By utilizing such procedures one can more reliably distinguish between the various stages and substages of disease. The inferior venacavogram, iv pyelogram, and lymphangiogram have not been helpful in nonbulky stage II disease. A persistently elevated HCG and/or AFP in a patient with testicular tumor treated by inguinal orchiectomy indicates stage II or III disease. Inferior venacavogram, computerized axial tomography scan, and lymphangiogram can be utilized to distinguish minimal stage II disease from maximal disease that may cause obstruction and/or invasion of the inferior vena cava and the ureters. The chest tomograms and biopsy of the left supraclavicular nodes may differentiate between stages II and III.

Surgical Management

Initially, a number of cases of testicular germ cell tumors may be seen by nonurologists; thus, it is important to reiterate the appropriate approach to a scrotal mass. First, unless the clinical history indicates otherwise, any scrotal mass in a young male should be suspect. Second, a serum sample should be obtained for AFP and HCG determination. Depending upon the history, physical examination, and/or tumor markers, an immediate exploration is advisable. The high inguinal incision allows exploration of the testis, with control of vascular and lymphatic channels. Direct inspection of the testis, after opening the tunica vaginalis testis, usually provides sufficient evidence to determine whether an orchiectomy

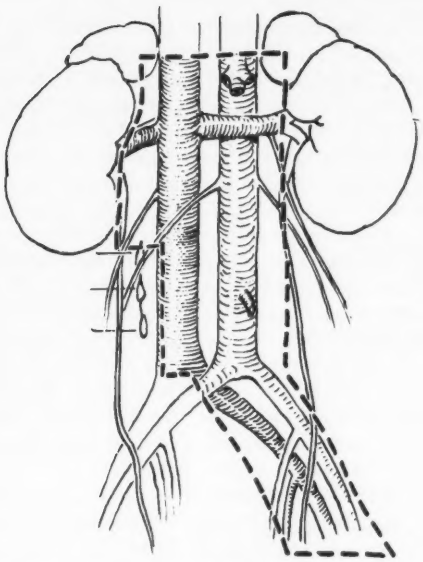


FIGURE 4.—Bilateral lymph node dissection for left-sided testis tumor.

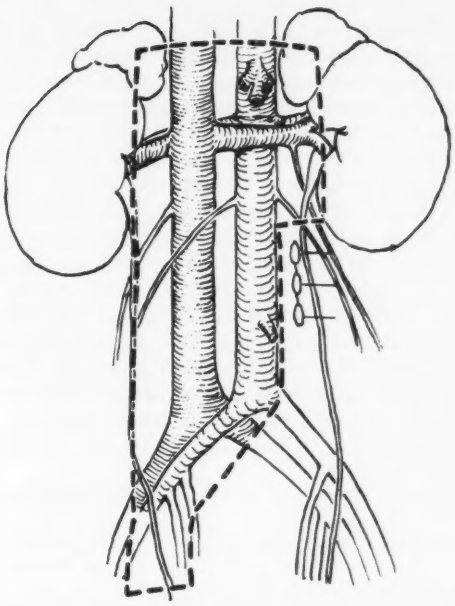


FIGURE 5.—Bilateral lymph node dissection for right-sided testis tumor.

should be performed. In an unusual case where a small hematoma or possibly an interstitial tumor and other disease can confuse the identification of the testicular lesion, the wound may have to be carefully packed and the testis and its covering must be opened away from the operative field. Testicular tumors can seed the scrotum and its separate lymphatic drainage. There is little justification for trans-scrotal biopsy of a scrotal mass. The radical orchiectomy specimen is examined by the pathologist and subsequent treatment depends on the cell type and stage of the cancer. The subsequent treatment of seminoma is radiotherapy and the subsequent treatment of pure choriocarcinoma is chemotherapy; embryonal carcinoma, immature teratoma, and teratocarcinoma patients undergo a retroperitoneal lymphadenectomy both for therapy and as part of the staging evaluation. Since the gonads embryologically are derived from tissue arising in the genital ridge near the vicinity of the renal blastema, the testicular tumors usually involve the aortic, caval, and renal lymphatic systems. The lymphatics of the right testis are drained to nodes located around the aorta from the renal vein to the common iliac node, including the lymphatics located around the region of the inferior mesenteric artery. Other sites of involvement that are relatively infrequently involved include the lower aortic nodes, and adequate lymphadenectomy must encompass the renal, aortic,, caval, and ipsilateral common, external, and internal iliac lymphatics (figs 4 and 5).

The conventional treatment for clinical stage I and II embryonal carcinoma and immature teratoma is retroperitoneal lymphadenectomy following radical orchiectomy (4). In a recent series of highly selected patients, Staubitz et al (5) reported 5-year disease-free survival rates of 87% and 83% respectively for patients with stage I and selected stage II nonseminomatous testicular tumors. Our data on survival also agree with these figures. Our current approach for the management of these tumors is shown in figure 6. The advent of tumor markers may well change the future treatment of testicular cancer (fig 7). One of the major problems in evaluating survival in patients with testicular tumors has been lack of prospective randomized clinical trials. The role of cytoreductive surgery in bulky stage II or III disease has been reported in nonrandomized studies (6).

National Cancer Institute Randomized Trial

To date, no prospective randomized trial has been performed to test whether effective chemotherapy can be enhanced by cytoreductive (debulking) surgery in patients with nonsurgically curable tumors. Although such an approach has scientific merit and the results from some treatment programs support its possible role, the ethical and technical aspects of such a study have been difficult to overcome. Testicular carcinoma patients who have multiple poor prognostic features are appropriate for such a study for the following reasons: (*a*) compared to

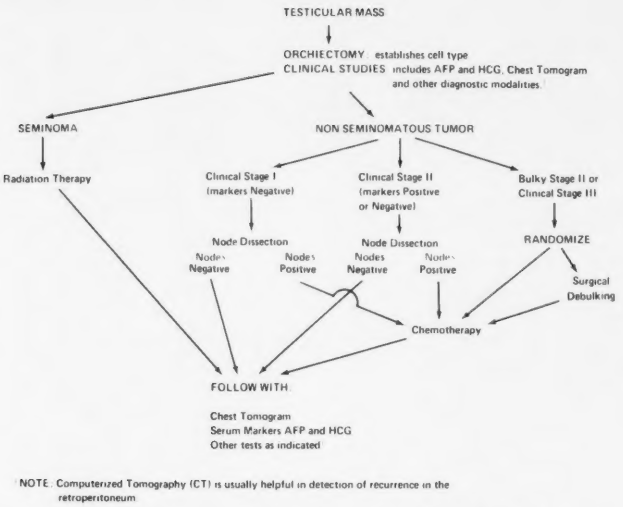


FIGURE 6.—Current management of testicular tumors.

patients with a minimal tumor burden their prognosis is very poor; (*b*) their ability to achieve a complete remission can be expected to significantly alter subsequent survival; (*c*) these patients are usually otherwise healthy, without other significant medical problems; (*d*) a major proportion of the metastatic disease can be surgically removed; (*e*) the known chemotherapeutic regimens have excellent antitumor activity; and (*f*) the levels of circulating tumor-associated proteins, ie, AFP and HCG, can be utilized to semiquantitate efficacy of cytoreductive surgery.

Based upon these considerations, the Surgical and Medicine Branches, National Cancer Institute, have begun a prospective randomized trial of cytoreductive surgery in stage III testicular carcinoma patients in which half of the patients will have debulking surgery of large retroperitoneal tumors (and of large isolated lung metastases) prior to chemotherapy (2,7–9). All patients are

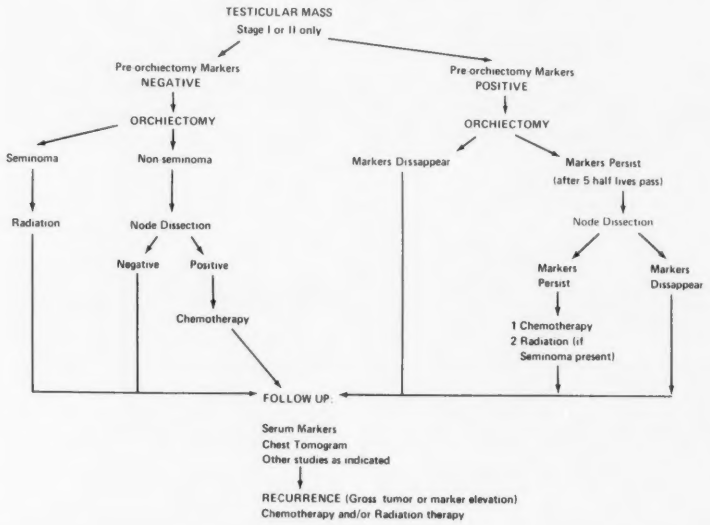


FIGURE 7.—A proposed protocol for research.

treated with a five-drug intensive chemotherapy regimen (basically a modification of the VAB-III [vinblastine, actinomycin D, bleomycin, cyclophosphamide, and *cis*-dichlorodiammineplatinum(II)] program). To date, 23 patients have been randomized in this study. Although it is too early to assess complete response rates and ultimate survivals, it is clear that: (*a*) cytoreductive surgery is technically feasible; (*b*) major portions of clinically evident macroscopic disease can be removed; (*c*) > 90% of the patients had tumors which produced HCG, AFP, or both, the circulating levels of the markers correlated with initial tumor burden, and with successful cytoreductive surgery the level of these markers decreased as much as 2 logs; and (*d*) with the aid of newer technology such as ultrasound, computerized axial tomography, and tumor markers, the incidence of "evolution" from a histologically malignant carcinoma to a mature teratoma, in patients who have not had surgery prior to chemotherapy, has been three of 11 patients. Since none of these patients have as yet remained disease-free for prolonged periods of time, it is not yet clear whether such "conversions" represent chemotherapy-induced differentiation of previously malignant clones of cells or selection of previously present benign clones of cells, or whether such benign tumors are indeed biologically benign. It is a fascinating scientific question and a perplexing patient-management problem. Results of this and analogous studies can be expected to help elucidate this question in the future.

Those aspects of testicular carcinoma which make these patients desirable candidates for studies of therapy for advanced disease also facilitate the introduction of adjuvant chemotherapy and/or radiotherapy trials; ie, (*a*) the discrepancies in histologic subtypes can be clarified by immunocytochemical studies; (*b*) early detection of residual postoperative disease can be achieved by the utilization of AFP and HCG assays; (*c*) improvements in staging techniques (ultrasound, computerized axial tomography scans, etc) and clarification of staging definitions can result in improved prognostication of potential disease behavior; and (*d*) effective therapy for advanced disease now exists as well as preliminary evidence that patients with small tumor burdens may be even more responsive. Because of all of these favorable features, a National Cancer Institute-sponsored extramural, nationwide, intergroup adjuvant chemotherapy and/or radiotherapy study for these young patients will be activated in the near future. A system comparing the clinical and surgicopathologic classification in stage I and II testicular cancer has also been proposed.[2] If successful, these studies may dramatically improve the prognosis for most patients with testicular neoplasms and can serve as a model for the integration of laboratory-based and clinically oriented research.

[2]Muggia FM, and Jacobs EM, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. Personal communication.

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Applications of Biologic Tumor Markers in Testicular Cancer

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SUMMARY

Quantitative measurement of serum alphafetoprotein (AFP) and human chorionic gonadotropin (HCG) by double antibody radioimmunoassays originally developed at the National Cancer Institute (Bethesda, Md) has been performed in 130 patients with seminoma and in 300 patients with nonseminomatous testicular cancer. Ten of 130 (7.7%) patients with seminoma, 99 of 152 (65%) patients with embryonal carcinoma with or without teratoma, and five of five (100%) patients with pure choriocarcinoma had an elevated level of HCG. None of the patients with seminoma or choriocarcinoma had an elevated level of serum AFP although elevated levels were present in 67% of the patients with embryonal carcinoma with or without teratoma. Either HCG or AFP was elevated in about 90% of the patients with clinically demonstrable embryonal carcinoma with or without teratoma and choriocarcinoma. These two markers have been helpful in staging, detecting recurrence, prognosis, and localization (by the α subunit of HCG) of a tumor. Utilizing immunocytochemical techniques, HCG has been localized in syncytiotrophoblastic component of choriocarcinoma. AFP has been localized in yolk sac tumor and embryonal carcinoma cells.

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During the past several years it has become apparent that testicular cancers synthesize special protein moieties which circulate in the serum and which may be particularly helpful in detecting tumors not otherwise clinically demonstrable (1). The recent development of sensitive methods to measure these proteins in the serum of patients with testicular cancer makes it possible to use these tumor products as markers for the presence of cancer (2). Among the proteins which have been found in the serum of patients with germ cell tumors of the testis are human chorionic gonadotropin (HCG) and alphafetoprotein (AFP). Sensitive and specific radioimmunoassays (RIA) have been developed by Vaitukaitis et al (3) for HCG and by Waldmann and McIntire (2) for AFP which are capable of measuring minute amounts of these markers in the serum. When these two serum markers are utilized together, they are the best serologic and cellular markers available for diagnosis, detection of early recurrence, and accurate staging, and may reflect the adequacy of treatment of testicular cancer.

After the development of new RIAs for these two markers at the National Cancer Institute (NCI) (Bethesda, Md), we undertook several prospective studies to prove the foregoing points. We have evaluated > 300 patients with nonseminomatous and 130 patients with seminomatous testicular tumors using HCG and AFP measurements. It is the objective of this paper to review our experience using these two markers in the diagnosis of testicular cancer.

MATERIALS AND METHODS

Patients

Four hundred and thirty patients with an initial diagnosis of seminoma, teratoma, embryonal carcinoma, teratocarcinoma (embryonal carcinoma and teratoma), or choriocarcinoma were entered in this prospective study at the NCI. After orchiectomy, the patients had extensive staging procedures which included detailed clinical

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[2]Serum alphafetoprotein and human chorionic gonadotropin were measured by Drs. T. A. Waldmann and K. R. McIntire.

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TABLE 1.—RIA of HCG and AFP as developed at the National Cancer Institute

Units and subunits	Half-life	Cross-reactivity*	Source	Normal (ng/ml)
AFP	5 days	None	Fetal liver Testis tumor	< 40
β-HCG	60 mins	None	Placenta Testis tumor	< 1
HCG	24 hrs	FSH, TSH, LH	Placenta Testis tumor	< 1
α-HCG	20 mins	FSH, TSH, LH	Placenta Testis tumor	< 2

*FSH = follicle-stimulating hormone; TSH = thyrotropic hormone; LH = luteinizing hormone.

information, hemogram, iv pyelogram, and bilateral pedal lymphangiogram. In some instances, scalene lymph node biopsy was performed on patients suspected of having metastatic disease. Before further therapy the patients were designated stage I, II, or III based on the clinical data including the serum markers. Most of the patients with seminomas were treated with radiotherapy; exceptions were patients with bulky metastatic retroperitoneal seminomas who had reductive surgery. Most of the patients with embryonal carcinoma and/or teratoma had surgical staging and therapy by aortocaval lymphadenectomy.

Selective Catheterization for Venous Sampling

With the catheterization technique by a percutaneous transfemoral approach, the venous blood samples were obtained from the ascending lumbar, azygos, superior vena cava, inferior vena cava, hepatic, renal, and iliac veins.

Radioimmunoassay of Serum Human Chorionic Gonadotropin and Alphafetoprotein

Double antibody RIAs were used for both markers. The assay for HCG used an antiserum against the β subunit of HCG and had a sensitivity of < 1 ng/ml; a serum level of > 1 ng/ml was considered elevated (3). The assay for AFP had a sensitivity of 5 ng/ml of serum; a serum level of > 40 ng/ml was considered abnormal (2) (table 1).

Immunoperoxidase Tissue Staining

The 4–6-μ serial sections of formaldehyde-fixed tumor were deparafinized in xylene and cleared in the usual fashion. These sections were incubated in a humid chamber for 60 minutes with rabbit anti-HCG (1:200) or anti-AFP (1:200) in 20% goat serum.

After washing, the sections were incubated for 60 minutes with goat anti-rabbit gamma globulin (1:200) conjugated with horseradish peroxidase in 20% goat serum. The sections were washed again and exposed to diaminobenzidine containing 0.05% H_2O_2 for 10 minutes. All the appropriate controls including sections exposed to normal rabbit serum instead of antisera to HCG or AFP were also included (4).

RESULTS

Frequency of Human Chorionic Gonadotropin and Alphafetoprotein in Testicular Cancer

Eleven of 130 patients with seminoma had elevated levels of serum HCG. One of these patients had an element of choriocarcinoma in a subsequent histologic section of the primary tumor. He developed aortocaval metastases that proved to be choriocarcinoma at the time of retroperitoneal lymphadenectomy. Sixty-four of 106 patients with embryonal carcinoma, 31 of 46 with teratocarcinoma, and five of five with choriocarcinoma had elevated levels of serum HCG. None of 130 patients with seminoma, 68 of 106 with embryonal carcinoma, 33 of 46 with teratocarcinoma, and none of five with pure choriocarcinoma had an elevated level of serum AFP. One of the 130 patients with seminoma had an elevated level of AFP (152 ng/ml). However, on serial sections we found an element of embryonal carcinoma. This patient received retroperitoneal radiation and lymphadenectomy and subsequent serum AFP measurements were within normal limits.

Seventy-two patients with embryonal carcinomas with or without teratomas were clinically considered stage I without considering prelymphadenectomy markers. Twenty of these patients were proven to have stage II disease after lymphadenectomy. In addition, three patients with negative lymph nodes (stage I) were proven to have lung metastases after a 3-month followup (stage

III), rendering an error rate of clinical staging of 32% (23 of 72 patients) when markers were not considered. When markers were considered, 57 of 72 patients were considered stage I, with 52 of these subsequently proven to have stage I, a staging error rate of 9%. Forty-six patients with embryonal carcinomas with or without teratomas were clinically staged as stage II without considering the markers. After retroperitoneal lymphadenectomy, 40 patients had positive lymph nodes and two of these were proven to have lung metastases 2–3 months after lymphadenectomy, resulting in accurate staging in 38 of 46 patients, a staging error rate of 17%. However, when the postlymphadenectomy markers were considered, 40 of 46 patients were stage II when markers were considered in staging and two patients with persistently elevated markers were eventually proven to have stage III, a staging error rate of 5% (38 of 40 patients).

Evolution

Four cases of conversion from embryonal carcinoma with or without teratoma to matured cystic teratoma were observed while the patients were receiving intensive chemotherapy including *cis*-dichlorodiammineplatinum(II). In three patients, the serum HCG or AFP did not return to normal and all three died in spite of intensive chemotherapy. In one patient both AFP and HCG returned to normal after intensive chemotherapy and a previously documented solid retroperitoneal tumor also became cystic. Resection of this tumor revealed cystic mature teratoma; the patient has been alive and free of tumor for the past 12 months.

DISCUSSION

HCG is a glycoprotein secreted by the normal placenta. It is normally found in the serum only during pregnancy. HCG has a molecular weight of 38,000 and is composed of two dissimilar subunits, α and β. The α subunit is the basic subunit of the pituitary glycoprotein hormones: luteinizing, follicle-stimulating, and thyrotropic. The β subunit, comprising two thirds of the molecular weight, is unique to the HCG and is distinct from the β subunits of luteinizing, follicle-stimulating, and thyrotropic hormones, particularly in the terminal 29 amino acids. The β subunit has been isolated, purified, and used to immunize rabbits to produce an antibody specific for HCG which does not cross-react with physiologic concentrations of the other glycoprotein hormones. The data from our laboratory indicate that 60% of the 300 patients with active nonseminomatous germ cell testicular tumors had elevated serum HCG levels (4). Elevated levels fell to normal with effective therapy. When an elevated level persisted or normal levels rose after initial therapy, recurrent tumor was invariably found. About 7.7% (ten of 130) of patients with testicular seminoma had elevated serum HCG levels (5).

Although the giant tumor cell which is occasionally found in "pure" seminoma is capable of secreting HCG, elements of choriocarcinoma and/or embryonal carcinoma must be looked for. This surely changes the therapeutic approach. The elevated serum AFP in patients with seminoma indicates an element of embryonal carcinoma that also changes the therapeutic approach. The reported cases of seminoma with elevated levels of HCG in the literature are lacking either serial sections or localization of cellular HCG. Therefore, we must be cautious in accepting them as "pure" seminoma. In this retrospective study it appears that the treatment of choice in patients with seminoma and elevated serum HCG (even in the absence of proven choriocarcinoma) is lymph node dissection and/or chemotherapy (5). However, the ultimate proof for this approach needs a prospective study as far as the survival rate is concerned.

In 1963, Abelev et al (6) demonstrated a specific α-1-globulin in the serum of embryonic and neonatal mice which was not found in the serum of adult mice. Subsequently, AFP was identified in the serum of mice bearing hepatocellular carcinoma and later in humans with hepatoma (7). Human AFP is a glycoprotein having a molecular weight of 70,000 and containing about 41% carbohydrate. It is produced in the liver, yolk sac, and gastrointestinal tract of the fetus. AFP is present in human fetal serum at a concentration of 3 ng/ml by the 12th week of gestation. At birth, the concentration is approximately 30 ng/ml and drops to much lower levels by 1 year of age; in normal adults it is found in concentrations of approximately 1–16 ng/ml. AFP has been clinically useful primarily as a diagnostic tool for hepatoma and certain other malignant diseases. Waldmann and McIntire (2), using a double antibody RIA for AFP, found an elevated level in 72% of 130 patients with hepatoma, in 23% of 44 patients with pancreatic carcinoma, in 18% of 91 patients with gastric carcinoma, and in 5%–7% of patients with colonic and bronchogenic carcinoma. Of particular interest was the finding by the same authors that 75% of 101 patients with testicular germ cell tumors had elevated levels of AFP. The discovery that testicular tumors so often produce AFP is a direct result of the development of a sensitive RIA; 71% of the elevated levels were < 3000 ng/ml, the lower limit of detectability of the previously used gel precipitation assay, and had gone undetected prior to the development of this sensitive RIA. In our series of 300 patients, about 70% of the patients had an elevated level of serum AFP. If an elevated level of AFP persisted after therapy, recurrent metastatic tumor generally was found.

Using these markers, we were able to improve the accuracy of clinical staging, to determine the presence of residual tumor after lymphadenectomy, to monitor the patient's response to chemotherapy, and to detect early recurrence of the tumor after treatment more accurately than using conventional studies.

About 90% of patients with nonseminomatous testicular tumors were found to have an elevated level of HCG, AFP, or both. The significance of these results is underlined by our finding that no patient without active tumor had elevated levels of these markers; that is, there were no false positives. The error rate in clinical staging was reduced by the use of these markers to 5%–15% (2). Moreover, the presence of an elevated level of HCG or AFP prior to definitive therapy (after orchiectomy had eliminated the primary tumor as a possible source of the elevated markers) had a substantial impact on the prognosis of the patient.

Prognostic Implications

Serial quantitative measurements have prognostic value in that they reflect the actual stage of the tumor. In patients with bulky disease receiving chemotherapy, we have observed a number of patients with a remnant of disease in spite of the return of the markers to normal. It appears that the chemotherapy has eradicated the clone of marker-producing tumor cells selectively and/or converted the tumor to a matured form. The prognostic significance of these phenomena is not completely clear at the present time. With the completion of an ongoing prospective randomized clinical trial at the NCI, the return of these markers to normal will be elucidated. If the elevated marker persisted after treatment, recurrent tumor invariably was found, often months before the patient was symptomatic or recurrence was detectable by any other clinical tests. Consequently, the markers proved to be sensitive indicators of the presence of otherwise undetectable metastases.

When following a patient with serial marker levels, the elevated level of a marker may be found before any clinically detectable tumor. The next question to answer in such a patient is the location of the recurrence. The value of the α subunit of HCG in localizing a tumor that is not detectable clinically by conventional clinical tests, including iv pyelogram, inferior venacavogram, and lymphangiogram, is illustrated in the following case. α-HCG has a short half-life (20 minutes) and may be used in the localization of metastases, especially in the retroperitoneal area where metastases are not readily detectable by the conventional clinical tests. In one patient, although peripheral α-HCG was within normal limits, selective catheterization of the right ascending lumbar vein revealed a twofold increase. Exploration of the right lumbar area revealed a 7 × 10–cm tumor with histologic features of an embryonal carcinoma containing an element of choriocarcinoma, explaining the persistent elevation of these two markers. We must emphasize that this localization would not be possible with serum AFP or HCG because of their prolonged half-lives (5 days and 24 hours respectively). With intensive chemotherapy the serum markers decreased to normal, but while patients were receiving maintenance chemotherapy the markers became elevated and exploration of the retroperitoneal area revealed multiple recurrences. The histologic features of the tumor were those of embryonal carcinoma containing syncytiotrophoblastic giant cell and yolk sac tumor (endodermal sinus tumor), a form of more differentiated embryonal carcinoma. Despite resection of these recurrences the markers did not decrease to the normal level. Autopsy revealed disseminated tumors, explaining the persistent elevation of these serum markers.

The tumors associated with the elaboration of AFP have been described under a variety of different designations, generally as teratocarcinoma, but recently there has been biochemical and immunohistochemical evidence to suggest that the endodermal sinus tumor, also known as yolk sac carcinoma or infantile-type embryonal carcinoma, is the specific neoplasm responsible for the synthesis of AFP (8). Elevated levels of HCG have generally been thought to reflect elements of choriocarcinoma, but there has been no systematic attempt to correlate the various patterns of germ cell tumors with the presence or absence of either AFP or HCG.

We have demonstrated AFP in mononuclear embryonal cells within embryonal carcinoma and endodermal sinus tumor. HCG was identified with syncytiotrophoblastic giant cells frequently in association with embryonal carcinoma, and rarely was identified with endodermal sinus tumor, seminoma, or the syncytiotrophoblastic component of choriocarcinoma. Eighteen of the 21 patients (86%) had elevated tumor markers in their serum; serum HCG alone was elevated in five (24%) and AFP was elevated in eight (38%). There was tissue localization of HCG in 12 of 13 patients (92%) with elevated serum HCG levels, while AFP was identified in the tumor in eight of 13 patients (53%) with elevated serum AFP levels (9). Thus, embryonal carcinoma (adult-type) is frequently associated with both AFP and HCG, endodermal sinus tumor is associated with AFP, choriocarcinoma is associated with HCG, and pure seminoma and teratoma are likely to be associated with either marker (table 2).

The syncytiotrophoblastic giant cells synthesize HCG and may be associated with pure seminoma, embryonal carcinoma, or yolk sac tumor. This may explain the occasional elevated HCG in the sera of these patients.

Also, the discordance between AFP and HCG could be explained on the basis of the findings that the cells producing HCG and AFP are different. During chemotherapy of a patient with elevated levels of serum HCG and

TABLE 2.—Cellular origin of testicular tumor markers

Cancer	AFP	HCG
Seminoma	–	–
Seminoma with STGC*	–	+
Embryonal carcinoma	+	–
Embryonal carcinoma with STGC	+	+
Yolk sac tumor	+	–
Yolk sac tumor with STGC	+	+
Mature teratoma	–	–

*Syncytiotrophoblastic giant cell.

AFP, one marker may return to normal although the other marker may still remain elevated. This may occur if some of the cells producing the respective marker are resistant to the given therapy.

With the aid of tumor markers, ultrasound, computerized axial tomograms, and immunochemical techniques, the incidence of evolution from a histologically malignant carcinoma to a mature teratoma is observed more frequently in patients with bulky tumor who did not have surgery prior to intensive chemotherapy. Since none of these patients have remained tumor-free for a prolonged period of time, it is not yet clear whether such conversions represent chemotherapy-induced differentiation of previously malignant clones of cells or, indeed, represent a true conversion to a benign tumor. We have extracted the HCG of 24-hour urine and detected its activity in patients with previously elevated serum HCG utilizing a new carboxyterminal RIA which is highly specific for β-HCG. Preliminary results indicate that utilizing this technique offers the potential for more sensitively monitoring the minute amount of residual tumor in guiding the therapy of patients with testicular cancer-producing HCG.

In conclusion, AFP and HCG, when utilized together, have been helpful in localizing and detecting recurrence, assessing tumor stage, and monitoring therapy for testicular cancer (10–12). The cellular localization of these markers by immunoperoxidase has also been an aid in understanding the pathogenesis and natural history of testicular cancer.

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Radiotherapy in the Management of Nonseminomatous Testicular Carcinoma

Eli Glatstein [1,*]

SUMMARY

Treatment of stage I and II nonseminomatous testicular carcinoma is reviewed. Retrospective studies show comparable cure rates following radiation therapy alone, surgery alone, or radiation therapy and surgery combined. For clinical stage I carcinoma, the long-term disease-free rates appear to be approximately 80%; for clinical stage II disease, the long-term disease-free rates appear to be approximately 60%. The development of effective combination chemotherapy for stage III disease suggests a need to rethink the approach for optimal management of stage II disease. Future clinical trials should be aimed at maximizing cure rates by attention to disseminated disease as well as by local control. Optimal management should also include some consideration of the long-term toxic effects of the various treatment modalities.

[Cancer Treat Rep 63:1649–1651, 1979]

Since the beginning of this century, survival rates for patients with testicular tumors have increased markedly. The basis for this improvement appears to lie in improving techniques of surgery, staging, radiotherapy, and, more recently, chemotherapy. Despite these improvements, however, the optimal methods of treating germ-cell neoplasms of the testis remain unclear; a remarkable dearth of prospective studies is apparent. Typically, the literature reveals series from single institutions comprised of numbers obtained by including patients over a 20–30-year period. Variations in pathologic classification, clinical and surgical staging, surgical technique, and types of radiotherapeutic management, as well as the introduction of effective chemotherapy, complicate meaningful analyses of these retrospective studies.

The purpose of this paper is to review concisely the role of radiation therapy in the treatment of local-regional nonseminomatous testicular carcinoma and to suggest future directions for clinical investigation.

Any discussion of treatment must begin with the natural history of the disease and staging. The important observations of the spread of nonseminomatous testicular carcinoma center around two major clinical features: (*a*) the remarkable ability of these diseases to spread and progress with extraordinary rapidity, and (*b*) the high frequency of retroperitoneal lymphatic involvement in virtually all patients who have disease spread beyond the testis itself.

The frequency of retroperitoneal lymphatic disease has led to the use of lymphangiography as a major tool in staging. While various staging systems depend upon surgical exploration for definition of the extent of retroperitoneal disease (1), bipedal lymphangiography has an 80%–90% accuracy rate (2–4) in experienced hands. The major point of discrepancy in lymphangiography concerns the presence of retroperitoneal disease in the area of the renal hilum, especially on the left, which does not opacify by bipedal lymphangiography, although it can be demonstrated experimentally by testicular lymphangiography (5,6). Presumably, computerized tomographic scans will be useful in this area, although their effectiveness is, as yet, unproven. Obviously, the lymphangiogram cannot reliably detect disease which is only microscopic in its dimensions. Nonetheless, in a practical sense, lymphangiography appears to be a valuable and reliable clinical means of staging the retroperitoneal region. When coupled with chest roentgenogram, whole-lung tomography, and, more recently, biochemical markers,

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relatively sophisticated staging can now be performed on patients.

In considering the results of radiation therapy for nonseminomatous testicular carcinoma, radiotherapy technique is as important for radiologists as surgical technique is for surgeons. Inclusion of data which antedate megavoltage treatment is clearly irrelevant. Modern megavoltage treatment, which began in this country in the late 1950s and early 1960s, achieved tumoricidal doses of 4500–5000 rads for nonseminomatous testicular carcinoma (7). Careful simulation of the patient in the treatment position by means of a simulator permits the bilateral periaortic node chains and ipsilateral iliac nodes to be encompassed. The superior margin of the field usually extends to the diaphragm; the inferior margin need not be below the inguinal ligament, unless there is a prior history of inguinal or scrotal surgery that may predispose to atypical lymphatic drainage. Obviously, if orchiectomy was performed after a scrotal incision, the scrotum itself must be included. Care must be taken to include the ipsilateral renal hilum within the periaortic and ipsilateral iliac field, typically referred to as the "hockey stick" field. With massive adenopathy, whole-abdominal treatment may be required. It is extremely important to set the patient up with iv pyelography with the patient assuming the precise treatment position in order to locate the kidneys exactly with respect to the high-dose radiation beam. If care is taken to localize the kidneys properly, then the risk of radiation-induced nephropathy is essentially eliminated.

In comparing the results of treatment in stage I and II nonseminomatous testicular carcinoma, careful attention must be given to the means of staging the patient. For stage I testicular carcinoma, 2-year disease-free survival rates following radical lymphadenectomy alone range from 80% to 90% (8–12). In most of these surgical series, staging is dependent upon the surgical findings, so that the stage I patients include only those with negative specimens. In patients with testicular carcinoma who undergo orchiectomy and a *negative lymphangiogram*, the 2-year disease-free survival rate following radiation therapy alone is comparable at 85% (8,9,13,14). It should be noted that in this group the only evidence of localized disease was a negative radiographic evaluation. The long-term success rates, however, are the same. In addition, retrospective studies (9,12,15) and one prospective study (16) failed to show any superiority for surgery and radiotherapy combined over radiotherapy alone in clinical stage I disease (ie, a negative lymphangiogram). Thus, clinical stage I testicular carcinoma appears to be a relatively favorable setting for treatment; reasonably adequate staging can be done radiologically. The results of radiotherapy alone, without surgical staging, appear to be comparable to those of radical surgery.

Since the risks of surgery are not inconsequential, and the problem of retrograde ejaculation following bilateral retroperitoneal lymph node dissection occurs in virtually all patients, questions arise concerning the role of surgery in clinical stage I disease. If the retroperitoneal lymph node dissection was negative, then it probably was not necessary and was of value for staging purposes only. If the retroperitoneal lymph node dissection revealed tumor, then it was in itself probably not adequate; most physicians today would recommend either additional postoperative radiation or postoperative chemotherapy. Certainly, there are long-term complications of periaortic irradiation; a finite risk of radiation enteritis, bowel obstruction, and bone marrow suppression is known to exist, but the frequency of such problems in the reported series varies from 5% to 10%. While many anecdotes exist regarding radiation complications in this setting, radiation-induced bowel injury occurs most frequently when radiation is used *after* prior surgical exploration and presumed small bowel fixation.

The role of adjuvant chemotherapy in the treatment of stage I testicular carcinoma is at present unclear. For major benefit to be demonstrated, the results would have to be spectacular, since the results of radiation therapy or surgery in stage I disease are really quite good. In addition, most of the present curative chemotherapeutic regimens for nonseminomatous testicular carcinoma are quite toxic and it would be difficult to justify their use at the present time since there are therapeutic alternatives which are comparatively simple and successful. Presently, the small proportion of patients with stage I disease who later relapse receive chemotherapy as soon as detectable disease is identified. While the precise salvage rate is unclear, preliminary data suggest that the salvage rate may be as high as 50% (17).

While the results of treatment by either irradiation or surgery in stage I disease appear to be acceptable, the results of treatment in stage II disease represent the major controversy in nonseminomatous testicular carcinoma. As previously noted, lymphangiography appears to have an accuracy rate of 80%–90% in the hands of experienced radiologists. Again, what constitutes stage II disease depends upon the nature of the staging techniques. While a few surgeons perform thoracolumbar incisions for testicular carcinoma (10), the vast majority explore the patient using an abdominal incision and a transperitoneal approach. A frozen section of lymph nodes above the renal hilum is typically obtained; if tumor is present at that site (or bulky, unresectable disease is detected), a dissection is usually not undertaken. As a result of this type of approach, patients who are finally subjected to a radical lymphadenectomy for stage II disease in most series consist of selected patients who do not have bulky disease and who do not have microscopic evidence of disease above the level of the renal arteries. The 2-year disease-free survival rate for surgical stage

II disease following lymphadenectomy alone is approximately 60%–70% (9,18), despite the fact that considerable selection is performed on these patients. The results of extensive radiotherapy alone in various retrospective studies with patients who have positive lymphangiograms (clinical stage II disease) show similar success rates of approximately 60% (7,9,13,14), although these studies clearly include patients who have disease which is above the level of the renal arteries and which could be "unresectable" if explored. Combinations of surgery and radiotherapy in retrospective studies (9,12,15) have also not shown any superiority over either approach alone.

A prospective study from the Walter Reed Army Medical Center (Washington, DC) in clinical stage II testicular carcinoma compared the results of radiotherapy alone to the results of radiotherapy plus surgery (16). Results at 3 years show no significant difference in these groups of patients, both showing approximately 80% disease-free rates. These figures are unusually high for stage II disease, but it should also be noted that these patients routinely had blind left scalene node biopsy; approximately 15%–20% of the patients who had a positive lymphangiogram had occult stage III disease in left neck nodes. Thus, this significant subgroup of patients was eliminated from the Walter Reed randomized study and was treated with chemotherapy.

Since the results in stage II disease in the best retrospective series show 60% cure rates following either radiotherapy or surgery, alone or in combination, oncologists who use all treatment modalities must acknowledge the dramatic improvements in stage III disease with chemotherapy alone (17,19,20). The duration of complete remissions following intensive chemotherapy using various regimens is still unclear; however, if the complete remission rate of 60% persists > 2 years after therapy is discontinued, then oncologists must rethink the therapeutic approach to stage II nonseminomatous testicular carcinoma. Conceivably, this group of patients could be well-managed with chemotherapy which is given until maximum responses are achieved. At that point, either surgery or radiation could be used, if thought to be necessary for residual disease. Indeed, this would be the makings of an excellent prospective clinical study, if all physicians caring for these patients could agree on such an approach.

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An Appraisal of the Role of Radiation Therapy in the Management of Nonseminomatous Germ-Cell Tumors of the Testis in the Era of Effective Chemotherapy

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SUMMARY

Patients with nonseminomatous germ-cell tumors of the testis can be divided into two broad groups. The first includes patients with negative lymphograms or small-volume metastases confined to the abdominal nodes. The overall cure rate with orchiectomy and nodal irradiation is 80%. A policy of early detection of relapse and treatment with chemotherapy is advocated. Adopting this approach, no deaths have occurred in this group of patients treated in 1976 and 1977, and only one (associated with acute myeloblastic leukemia) occurred in 1975. The second group consists of patients with other stage categories who receive chemotherapy as initial treatment, followed in stage II and III and selected stage IV patients by radiation therapy to sites of initial involvement and surgery. Preliminary experience has shown this to be a practicable and promising approach. The prognosis for stage IV patients depends upon metastatic site and volume; in those patients with limited lung disease 80% are surviving disease-free.

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Historically, the classic management of stage I and II (fig 1) testicular tumors has been by orchiectomy and radical node dissection in the United States and by orchiectomy and radiation therapy in Great Britain. With the development of more effective chemotherapy it is important to reconsider the potential roles of both surgery and radiation in overall management. The role of radiation therapy will be considered from this point of view under the following headings.

1. Assessment of the response of nonseminomatous germ-cell tumor metastases to radiation therapy.
2. Consideration of the results of treatment, patterns of treatment failure, and morbidity from orchiectomy and nodal irradiation as primary management.
3. Patient tolerance to surgery and chemotherapy before or after radiation therapy.

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RESPONSE OF NONSEMINOMATOUS GERM-CELL TUMOR METASTASES TO RADIATION THERAPY

Subclinical Metastases

For many years, the clinical stage I patient has been managed, following orchiectomy, by irradiation of the ipsilateral pelvic nodes and the para-aortic lymph node area extending to the lower dorsal region. The overall results in this group of patients have been good (vide infra), but it is pertinent to consider whether this is a reflection of the absence or low frequency of metastases in retroperitoneal nodes of the lymphogram-negative patient, or whether the high disease-free survival rate can be attributed to the eradication of small-volume deposits of tumor. Table 1 summarizes details on 316 patients with negative lymphograms who subsequently had histologic examination of the nodes. Approximately 25% of the patients had pathologic evidence of tumor despite the negative lymphogram. In an analysis of the sites of relapse following irradiation for early-stage disease, we have previously reported that relapse in the irradiated abdominal nodes is a rare event (1), and it may therefore be concluded that subclinical deposits of tumor are eradi-

I. Lymphogram negative, no evidence of metastases.

II. Lymphogram positive, metastases confined to abdominal nodes, 3 subgroups are recognized:

A. Maximum diameter of metastases < 2 cm.
B. Maximum diameter of metastases 2–5 cm.
C. Maximum diameter of metastases > 5 cm.

(The distinction between B and C does not at present influence the choice of therapy and patients are not analyzed separately in this report. The distinction is retained since it is felt that prognostic differences are likely to become apparent in due course.)

III. Involvement of supradiaphragmatic and infradiaphragmatic lymph nodes. No extralymphatic metastases.

Abdominal status: A, B, and C, as for stage II above.

IV. Extralymphatic metastases.

Abdominal status: 0 = lymphogram negative; A, B, and C, as for stage II above.

Lung status:

$L_1 = \leq 3$ metastases.
L_2 = multiple metastases, $\leq$ 2-cm maximum diameter.
L_3 = multiple metastases, > 2-cm diameter.

Liver status: H+ = liver involvement.

FIGURE 1.—Staging classification.

cated by nodal irradiation. Further evidence on this point will come in due course from the analysis of α-fetoprotein and human chorionic gonadotropin levels in relation to orchiectomy and irradiation in the clinical stage I patient. Such a study is complicated by the need for sequential marker measurements following orchiectomy (with, if possible, a pre-orchiectomy sample), since clearance of the marker produced by the primary tumor may be incomplete by the time irradiation is commenced. Thus, a single sample may not reflect marker production by subclinical metastases. The prognostic significance of marker production per se, and of elevated titers due to metastases rather than persistence of marker from the primary tumor, needs to be distinguished and work in this area is in progress. Persistent elevation of markers after radiation therapy is of course a clear indication of the presence of residual tumor.

Clinically Detectable Tumor Metastases

Lymph Node Metastases

The incidence of histologically negative nodes in lymphogram-positive patients undergoing surgery is low,

TABLE 1.—Occult metastases from testicular teratoma in negative-lymphogram patients

Authors (ref No.)	No. of patients with— Negative lymphograms	Positive histology
Fein and Taber, 1969 (10)	30	10
Wallace and Jing, 1970 (11)	49	8
Hussey et al, 1977 (12)	73	13
Maier and Schamber, 1972 (13)	24	6
Hultén et al, 1973 (14)	16	4
Jonsson et al, 1973 (15)	10	2
Safer et al, 1975 (16)	21	3
Durant and Barrat, 1977 (17)	14	6
Kademian and Wirtanen, 1977 (18)	16	4
Lasser et al, 1977 (19)	11	4
Cook et al, 1965 (20)	12	4
Seitzman and Halaby, 1964 (21)	12	6
Storm et al, 1977 (22)	28	10
	316	80 (24.7%)

and even allowing for the variability in criteria for lymphographic interpretation, it is clear that the large majority of patients with clinical stage II disease may be assumed to have nodal metastases. Approximately 50% of patients in stage II are cured by orchiectomy and radiation therapy (vide infra). In a retrospective analysis we drew attention to the dependence of therapeutic response on metastatic volume (2). In this study, patients with abdominal node metastases < 2 cm in maximum diameter had a low local recurrence rate and an overall cure rate of 80%, whereas in patients with more bulky tumor deposits only 35% survived and local recurrence was invariably associated with treatment failure. These observations, made on a small number of patients, indicate that the clinically apparent small-volume tumor deposit can be eradicated but that radiation therapy alone is inadequate treatment for bulkier disease. Maier and Mittemeyer (3) reported a 3-year disease-free survival in nine of 11 stage II patients following radiation therapy and noted that the smaller-volume metastases tended to fall into this group. Further indirect evidence of the response of nodal deposits to radiation may be gained from the results of those centers employing pre-operative irradiation before node dissection. Thus, Klein and Maier (4), comparing experience at the Walter Reed (Washington, DC) and M. D. Anderson (Houston, Tex) Hospitals, reported that the incidence of positive nodes was reduced in patients receiving 2500–3000 rads before surgery. These observations are summarized in table 2.

Pulmonary Metastases

There is little documented evidence defining the response of lung metastases to radiation. In general terms, clinically apparent metastases receiving lung tolerance doses of the order of 2000 rads regress and regrow with

TABLE 2.—Results of node dissection with or without preoperative irradiation (from ref 4)

Clinical stage	Preoperative radiation therapy*	Incidence of histologically positive nodes at dissection†	
		M. D. Anderson Hospital	Walter Reed Hospital
I	–	18/106 (17%)	6/24 (25%)
	+	0/5	1/30 (3%)
II	–	20/22 (91%)	32/35 (91%)
	+	15/28 (54%)	11/21 (52%)

*– = therapy not given; + = therapy given.
†No. of patients with positive nodes/No. of dissections performed.

a volume growth delay of 2–3 months. Van der Werf Messing (5) has reported a 40% cure rate in a small group of highly selected patients with a limited number of metastases who received 3000 rads to the lungs and booster doses to identifiable deposits. Since solitary pulmonary metastases are commonly associated with subclinical deposits in the lungs, this observation adds some support to the observations made for stage I disease that small aggregates of tumor appear responsive to moderate doses of radiation.

RESULTS OF ORCHIECTOMY AND RADIATION THERAPY

The results for stage I and small-volume stage II (IIA) patients are good, whereas the results for stage IIB, III, and IV patients have hitherto been poor (table 3). These results are comparable to those reported elsewhere in the literature. Thus, Van der Werf Messing (5) reported a 90% cure rate for clinical stage I patients and 45% for node-positive patients with nonpalpable disease. Maier and Mittemeyer (3) reported that 86% of stage I patients and 82% of a small group (11) of stage II patients were disease-free at 3 years.

Figure 2 shows survival curves for the two main subgroups in the Royal Marsden series. The difference between the early-stage groups (I and IIA) and the later-stage groups (IIB, IIC, and III) is significant ($P < 0.01$).

The pattern of relapse between these two groups shows important differences. As mentioned above, relapse in the early-stage group occurs predominantly in the lung and supradiaphragmatic nodes but rarely occurs in the abdomen. Conversely, in patients with advanced nodal involvement extralymphatic extension is commonly associated with relapse within an irradiated area (1).

Toxicity

The immediate and long-term sequelae of nodal irradiation have, in our experience, been minimal when patients are given doses of the order of 4000 rads in 4–5

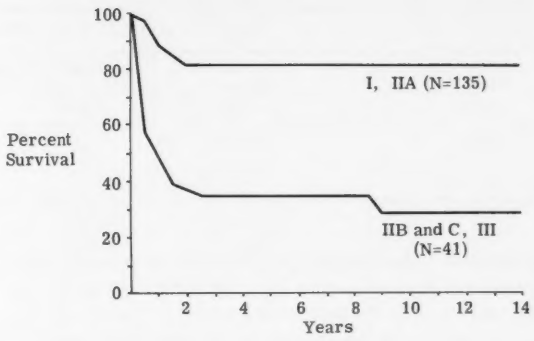

FIGURE 2.—Survival of patients with malignant testicular teratoma according to stage.

weeks and are treated by anterior and posterior fields with 6-MeV photons from a linear accelerator. If doses of 4500 rads are exceeded and particularly if large volumes are irradiated, the probability of small bowel damage will of course increase. Irradiation of both the abdominal and supradiaphragmatic nodes inevitably involves the inclusion of a large volume of bone marrow. If the patient is managed by radiation alone this does not pose any problems, but if chemotherapy is to be associated with radiation therapy, careful consideration is required. This aspect of treatment is discussed below.

As far as testicular function is concerned radiation therapy has no effect on potency. If a radical orchiectomy with high-cord ligation has been carried out, the scrotal sac and inguinal nodes can be omitted from the radiation field, and the testes can be protected with lead cups. In these patients, which constitute the majority, fertility can also be preserved (6,7).

PATIENT TOLERANCE TO CHEMOTHERAPY AFTER RADIATION THERAPY

As outlined below it is the policy of this unit to advocate the use of chemotherapy[2] as initial therapy for stage IIB, IIC, III, and IV patients. In stage I and IIA patients, however, where 80% of the patients are cured by orchiectomy and radiation therapy together with recent, more effective chemotherapy, it is argued that systemic treatment should be deferred until relapse occurs.

Of 15 patients treated with vinblastine-bleomycin (8) for relapse after radiation therapy, in most cases given at the referring center, seven are alive at 12–29 months (average followup, 18.7 months). This form of therapy is

[2]Between 1975 and mid-1978 the vinblastine-bleomycin regimen described by Samuels et al (8) was employed. More recently, *cis*-platinum has been added and the schedule proposed by Einhorn and Williams (9) has been adopted.

TABLE 3.—Disease-free survival in stage I–III nonseminomatous germ-cell tumors of the testis: The Royal Marsden Hospital, 1962–1977

Clinical stage	No. of patients with disease-free survival/No. treated					
	1962–1972	1973	1974	1975	1976	1977
I and IIA	74/91 (81.3%)	9/11 (81.8%)	11/14 (78.6%)	12/13 (92.3%)	11/11 (100%)	17/17 (100%)
IIB, IIC, and III	5/19 (26.3%)	0/4	1/3 (33%)	5/14 (28.9%)	3/7 (42.8%)	4/4 (100%)

practicable if good supportive care can be assured, particularly if irradiation has been limited to the abdomen and, as is usually the case, if an interval of several months has elapsed between completion of irradiation and detection of relapse. The majority of these patients were referred with advanced disease either because relapse was not detected early or because radiation therapy had been inappropriately employed for bulky lymph node metastases.

TABLE 4.—Occult supraclavicular node metastases in teratoma patients with abdominal node metastases (stage II)

Authors (ref No.)	No. of patients sampled	No. with positive histology
Buck et al, 1972 (23)	23	3
Donohue et al, 1977 (24)	14	3
	37	6 (16.2%)

CONCLUSION AND GENERAL STRATEGY

Early-Stage Patients (I and IIA)

Radiation therapy is capable of eradicating small-volume metastases in clinical stage I and IIA patients. Treatment failure in 20% of these patients is due to extralymphatic metastases present ab initio. Marker studies may define this group for treatment as stage IV patients from the outset. Until such markers can be detected, a policy of deferred chemotherapy for early relapse is advocated.

As shown in table 3, of 41 stage I and IIA patients treated in 1975, 1976, and 1977 there has only been one death, although there were five relapses. It is clear that a proportion (perhaps 70%–75%) of patients with stage I disease are node-negative and need no treatment (either surgical or radiotherapeutic) to the abdominal nodes. The choice between node dissection and irradiation should be based upon a consideration of relative toxicity, both of the treatment itself and from its interaction with systemic therapy, which may become necessary in 20% of patients. Radiation therapy correctly applied is noninvasive and, if limited to the abdominal nodes, has not in our experience seriously compromised subsequent chemotherapy.

Elective Supradiaphragmatic Irradiation

It has hitherto been our policy to irradiate the mediastinum and supraclavicular nodes in stage IIA patients. The incidence of occult nodal metastases is probably small as shown in two limited series of patients summarized in table 4. Once spread has occurred along the thoracic duct to the cervical nodes, the probability of extralymphatic dissemination is high and irradiation to the mediastinum and neck would achieve nothing but would, by irradiating an appreciable volume of marrow, compromise subsequent chemotherapy. It is felt, for this reason, that supradiaphragmatic irradiation should not be undertaken electively in this group of patients.

Later-Stage Patients (IIB, IIC, and III)

In this group chemotherapy is employed with two aims: the elimination of small-volume extralymphatic metastases and the reduction in volume of bulky masses of metastatic tumor. On the assumption that residual tumor may be present in initially large nodal deposits, chemotherapy is followed by irradiation to sites of initial involvement. The hypothesis being tested is that this combination may in itself be an effective method of treatment. In order to assess the performance of the drug-radiation combination, residual masses in the retroperitoneal area are excised 6 weeks after completion of radiation therapy. Preliminary results obtained with this approach have been reported recently (25). To date, 13 patients receiving chemotherapy and radiation therapy have been explored. Only two (15%) have had histologic evidence of residual tumor (26).

The protocol is summarized in figure 3 and includes stage IV patients which are discussed briefly below.

Stage IV Patients

In the patient with extralymphatic metastases prognosis has, until recently, been very poor. In patients treated prior to 1976 only 10% were cured, whereas results in the more recent group indicate that survival fig-

TABLE 5.—Influence of metastatic volume on response to vinblastine-bleomycin in testicular teratoma: The Royal Marsden Hospital, 1975–1977

Site of metastasis	Clinical substage	No. of patients treated	No. with complete remission
Abdominal nodes	A	3	3 (100%)
	B	6	2 (33%)
	C	18	1 (5.6%)
Lung	L_1 and L_2	22	16 (72.7%)
	L_3	20	5 (25%)

ures have improved appreciably. In stage IV patients a range of problems may be defined. There is a volume-related response to chemotherapy (table 5). This observation, taken in conjunction with our previous observations on the influence of tumor volume on radiation response, is the basis for the subdivision of stage IV proposed in the staging classification (fig 1). The combined approach of chemotherapy-radiation-surgery is employed in patients with small-volume lung metastases (L_1 and L_2) and bulky nodal metastases. Patients with massive lung disease (L_3) and/or hepatic involvement are treated with chemotherapy alone. Of 21 previously untreated patients with stage IV L_1 or stage IV L_2 disease, 17(81%) are disease-free compared with only three of 18 (17%) of stage IV L_3 and stage IV H+ patients (25).

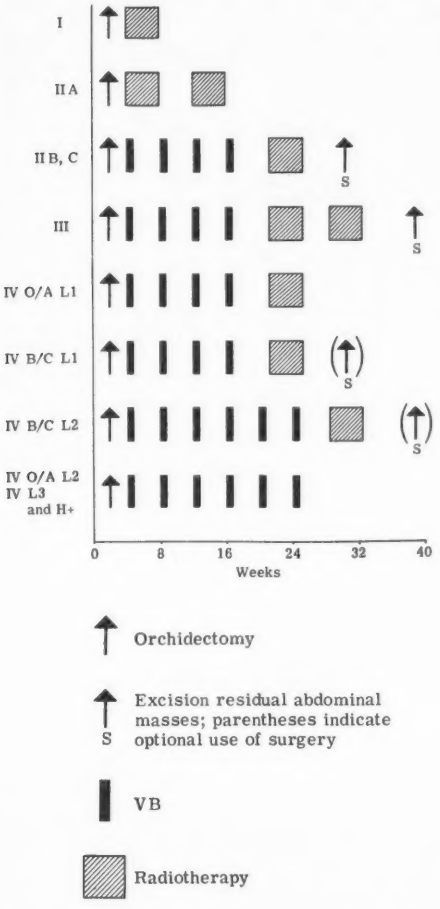

FIGURE 3.—Treatment protocols for testicular teratoma. VB = vinblastine/bleomycin.

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Combination Chemotherapy With *cis*-Dichlorodiammineplatinum(II) in Disseminated Testicular Cancer[1]

Lawrence H. Einhorn[2,*]

SUMMARY

Combination chemotherapy with *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) has been used at Indiana University since August 1974. *cis*-Platinum (20 mg/m^2 × 5 days every 3 weeks for three to four courses), vinblastine (0.4 mg/kg every 3 weeks for four courses), and bleomycin (30 units weekly for 12 weeks) were utilized as remission induction therapy until September 1976. This regimen produced 30% partial and 70% complete remission (CR) rates among 47 evaluable patients. Furthermore, an additional five patients (11%) were rendered disease-free following surgical removal of residual disease. Thirty-two (68%) of these patients remain alive, and 28 (60%) remain alive and disease-free. Twenty-six of these 28 patients have now been continuously disease-free for 2+ to 4+ years and are receiving no chemotherapy. From September 1976 to June 1978, an additional 79 patients were included in a three-arm randomized prospective study with *cis*-platinum, vinblastine, and bleomycin with or without Adriamycin, using different doses of vinblastine. Fifty-three patients (67%) achieved CR; an additional ten patients (13%) were rendered disease-free with surgical removal of residual localized disease. Only seven of these 63 patients (11%) have relapsed. One patient died due to a pulmonary embolus 3 months after surgical resection for residual embryonal carcinoma. Thus, 55 patients (70%) remain disease-free. Sixty-seven (85%) of these patients remain alive. Thus far, there has been no significant difference in CR rate or survival in any of the three arms. *cis*-Platinum, vinblastine, and bleomycin combination chemotherapy regimens consistently produce a 60%–70% CR rate; 10%–15% of the patients will be rendered disease-free following surgical removal of localized residual disease after significant chemotherapy-induced partial remission. The relapse rate remains low (10%–20%). We feel this represents a major advance in the management of disseminated testicular cancer.

[Cancer Treat Rep 63:1659–1662, 1979]

Although testicular cancer accounts for only 1% of all malignant tumors in males, it ranks first in incidence of cancer deaths in the 25–34-year age group (1). Thus, cancer of the testis has a significant impact on the social, economic, and emotional status of this young population.

In 1960, Li et al (2) introduced the first major program of chemotherapy for advanced testicular cancer with the combination of actinomycin D, chlorambucil, and methotrexate. Subsequent studies confirmed a 50%–70% response rate, which included 10%–20% complete remission (CR) rates (3,4). The past 15 years have also seen the development of many new agents with substantial activity, notably vinblastine (5), bleomycin (6), and mithramycin (7). These single-agent studies demonstrated not only that a CR could be obtained in disseminated testicular cancer, but that approximately half of these CRs were permanent cures (3–5,7). Most relapses occurred within 2 years after starting chemotherapy.

Combination chemotherapy has produced excellent long-term CRs in other chemosensitive tumors such as Hodgkin's disease (8). Similarly, most recent attempts at improved chemotherapy in testicular cancer have been

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with combination chemotherapy. One of the most widely used combinations has been vinblastine plus bleomycin (9). *cis*-Dichlorodiammineplatinum(II) (*cis*-platinum) is one of a group of coordination compounds of platinum identified by Rosenberg et al (10) that strongly inhibits bacterial replication. This agent has significant activity in refractory advanced testicular cancer, and furthermore, it is ideal for combination chemotherapy because of its relative lack of myelosuppression (11). It is our feeling that *cis*-platinum is *the* most effective agent in the treatment of disseminated testicular cancer.

MATERIALS AND METHODS

One hundred and twenty-nine patients with disseminated testicular cancer were included in these studies. Fifty patients were treated with "standard" *cis*-platinum, vinblastine, and bleomycin therapy (fig 1), and these results have previously been reported (12). Three patients were considered unevaluable because of early death (due to massive carcinoma) prior to completion of 2 weeks of therapy. Initially, bacillus Calmette-Guérin (BCG) immunotherapy was given if CR was achieved in an attempt to augment host cell-mediated immunity and prolong the duration of CR. In recent years, we have questioned the value of adding BCG to the treatment programs of such patients, and during the past 2 years, we have not used any form of immunotherapy. Since our relapse rate remains at a very low level despite cessation of BCG, it appears unlikely that BCG contributed any therapeutic advantage. Vinblastine was given 6 hours prior to bleomycin in an attempt to synchronize tumor cells for maximal destruction by bleomycin. However, this

(1) cis-Platinum: 20 mg/m^2 given iv (during 15 mins) daily for 5 days, once every 3 wks for 3 courses

   a. A 4th course is given if CR is not achieved with 3 courses
   b. Saline hydration is started at least 12 hrs prior to cis-platinum and continued throughout the 5-day course of cis-platinum at a rate of 100 ml/hr of normal saline

(2) Bleomycin: 30 units given iv weekly for 12 wks

(3) Vinblastine: 0.2 mg/kg given iv daily for 2 days (0.4 mg/kg) every 3 wks

   a. Vinblastine is given 6 hrs prior to bleomycin
   b. Maintenance vinblastine (0.3 mg/kg every 4 wks) is started after completion of bleomycin and continued for 2 yrs

FIGURE 1.—Standard *cis*-platinum, vinblastine, and bleomycin therapy.

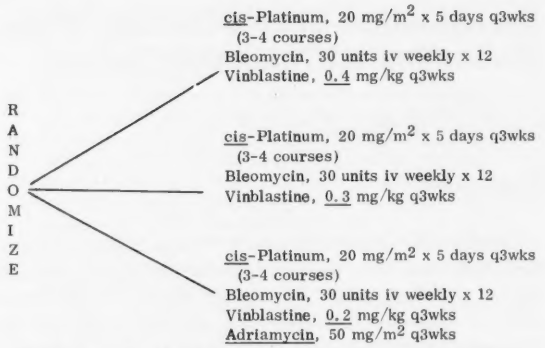
RANDOMIZE
cis-Platinum, 20 mg/m2 x 5 days q3wks
(3-4 courses)
Bleomycin, 30 units iv weekly x 12
Vinblastine, 0.4 mg/kg q3wks
cis-Platinum, 20 mg/m2 x 5 days q3wks
(3-4 courses)
Bleomycin, 30 units iv weekly x 12
Vinblastine, 0.3 mg/kg q3wks
cis-Platinum, 20 mg/m2 x 5 days q3wks
(3-4 courses)
Bleomycin, 30 units iv weekly x 12
Vinblastine, 0.2 mg/kg q3wks
Adriamycin, 50 mg/m2 q3wks

FIGURE 2.—Randomized study of *cis*-platinum, bleomycin, and vinblastine with or without Adriamycin. After completion of 12 wks of bleomycin, maintenance therapy on all 3 arms consists of vinblastine at a dose of 0.3 mg/kg monthly for 2 yrs.

synchronization has also been abandoned recently, and we now give vinblastine and bleomycin at the same time.

Seventy-nine patients were treated from September 1976 to June 1978 with the randomized study shown in figure 2.

A partial remission (PR) was defined as a decrease of $\geq$ 50% in the sum of the products of diameters of all measurable lesions. A CR was defined as the complete disappearance of all clinical, radiographic, and biochemical evidence of disease, including normal whole-lung tomograms, serum human chorionic gonadotropin, alphafetoprotein, and normal abdominal computerized axial tomographic scan and ultrasound. Anything less was not called a CR.

RESULTS

Fifty patients were treated with *cis*-platinum, vinblastine, and bleomycin from August 1974 to September 1976. Since these results have previously been reported (12), only the updated results will be discussed.

The CR rate was 70% (33 CRs among 47 evaluable patients). Furthermore, five patients (11%) were rendered disease-free following surgical removal of residual disease. Three of these five patients were found to have histologically converted from embryonal carcinoma to mature teratoma and remain disease-free 2½–3½ years postoperatively. Both patients who had persistent embryonal carcinoma surgically resected relapsed while receiving vinblastine maintenance therapy and died shortly thereafter.

Only six of these 33 patients with chemotherapy-induced CRs have relapsed. One patient relapsed while receiving vinblastine maintenance therapy; subsequent therapy with Adriamycin, bleomycin, and vincristine was unsuccessful, and he died shortly after relapse. One patient refused maintenance therapy and was temporarily

lost to followup; he returned in a moribund condition with massive pulmonary metastases and died within 24 hours of re-admission. The other four patients relapsed while receiving vinblastine maintenance therapy. Although three of these four patients achieved a second remission with re-induction with *cis*-platinum, Adriamycin, vincristine, and bleomycin, they all subsequently relapsed. Interestingly, two of these patients achieved a third CR with *cis*-platinum plus the epipodophyllotoxin derivative, VP-16-213. It is noteworthy that five of these six relapses occurred within 9 months of chemotherapy, and the only other relapse occurred at 17 months.

The toxicity of this regimen has previously been described (12). The major serious toxic effect was severe granulocytopenia. Eighteen patients required hospitalization for presumed sepsis. Seven of these patients had documented sepsis, one of whom died as a result.

cis-Platinum, Vinblastine, and Bleomycin With or Without Adriamycin

Despite excellent CR rates and survivals with the previously described *cis*-platinum, vinblastine, and bleomycin regimen, we have been concerned about the toxicity. Although *cis*-platinum is potentially nephrotoxic, this has only rarely been a clinical problem when vigorous saline hydration is used. Similarly, clinically significant bleomycin pulmonary fibrosis is an uncommon complication. Thus, the major serious toxicity is related to high-dose (0.4 mg/kg) vinblastine. Myalgia, constipation, and paralytic ileus were all troublesome side effects, but severe granulocytopenia and potential sepsis were the most worrisome effects. Since there are no firm data indicating that such high doses of vinblastine are required for optimal therapeutic effect, we started a randomized prospective trial in September 1976, comparing our standard *cis*-platinum, vinblastine (0.4 mg/kg), and bleomycin regimen with the same regimen using a lower dose (0.3 mg/kg) of vinblastine. Because of encouraging results achieved with *cis*-platinum and Adriamycin in patients who progressed while receiving vinblastine and bleomycin (13), it was decided to add a third arm to the randomized prospective study evaluating Adriamycin as first-line chemotherapy in combination with *cis*-platinum, vinblastine, and bleomycin (fig 2). Patients in all three arms received identical maintenance therapy (vinblastine at a dose of 0.3 mg/kg every 4 weeks for a total of 2 years).

Seventy-nine patients have been entered in this randomized prospective study, and the results are shown in table 1. The CR rate (67%) and the surgical resection rate for localized residual disease (13%) were remarkably similar to those in our original *cis*-platinum, vinblastine, and bleomycin study (70% and 11% respectively). The relapse rate remains low. There have been no drug-related deaths in these 78 patients; however, one patient died of a pulmonary embolus 3 months after laparotomy for removal of residual embryonal carcinoma. An autopsy failed to reveal any evidence of gross or microscopic carcinoma. Fifty-five patients (70%) remain continuously disease-free and 67 patients (85%) remain alive.

DISCUSSION

Since the original report by Li et al (2) in 1960 demonstrating activity of chemotherapy in disseminated testicular cancer, there have been numerous clinical trials evaluating a variety of drugs in testicular cancer. One point that is clear from these studies in the 1960s is that > 50% of these early CRs were apparent cures, and the majority of relapses occurred during the first year of chemotherapy with a smaller number of relapses occurring during the second year (2–5,7).

During the past 5 years, we have treated 129 patients with disseminated testicular cancer. One hundred and one of these patients have achieved a disease-free status (86 patients with chemotherapy alone and 15 with chemotherapy plus surgery). Only 15 of these patients have relapsed; all relapses occurred within 1 year of initiation of chemotherapy except for one relapse that occurred at 17 months.

Our experience with combination chemotherapy in patients with advanced testicular cancer allows us to suggest the following guidelines:

TABLE 1.—*cis*-Platinum, vinblastine, and bleomycin (PVB) with or without Adriamycin

	PVB (vinblastine = 0.3 mg/kg)	PVB (vinblastine = 0.4 mg/kg)	PVB + Adriamycin	Totals
No. of patients	27	26	26	79
No. of CRs	16 (59%)	18 (69%)	19 (73%)	53 (67%)
No. of PRs	10 (37%)	8 (31%)	6 (23%)	24 (30%)
No. of disease-free patients after surgery	3 (11%)	5 (19%)	2 (8%)	10 (13%)
No. of relapses*	2 (10%)	4 (17%)	1 (5%)	7 (11%)
No. of patients still alive	22 (81%)	24 (92%)	21 (81%)	67 (85%)
No. of patients with no evidence of disease	17 (63%)	18 (69%)	20 (77%)	55 (70%)

*Applies to patients in CR or to those patients rendered disease-free following surgical removal of residual disease.

(*a*) The goal of therapy in all patients should be a CR; if a clinical CR is not achieved after three courses of *cis*-platinum in a responding patient, then a fourth course should be given. We have not found any value in exceeding four courses.

(*b*) If a clinical CR has not been achieved with chemotherapy, surgical excision of residual disease should be performed, if feasible. The two clinical situations where this occurs are:

1. Complete disappearance of pulmonary metastases except for a solitary residual nodule or nodules confined to a single lobe of the lung; wedge resection is the preferred surgical approach.
2. Persistent abdominal disease. It has been our policy to do an exploratory laparotomy with removal of any residual tumor in all patients who initially presented with disseminated disease and achieved complete eradication of pulmonary metastases (confirmed by whole-lung tomograms) but still had evidence of persistent abdominal disease (palpable abdominal mass or persistently abnormal abdominal computerized axial tomographic scan or ultrasound). We have not felt it necessary to do a laparotomy or retroperitoneal node dissection in any of the other patients in this series, despite the fact that many of them had evidence of abdominal disease initially.

(*c*) The major toxic effect with this therapeutic regimen has been the significant leukopenia and potential granulocytopenic sepsis secondary to vinblastine. However, preliminary results indicate that lower doses of vinblastine will produce equivalent therapeutic results with less toxicity. Any patient with prior irradiation should have a 25% dose reduction of vinblastine.

(*d*) It has been our practice to hospitalize any patient with a temperature of $> 38.3°C$ and a granulocyte count of $< 1000/mm^3$, to obtain appropriate cultures, and to institute broad-spectrum antibiotic therapy with a cephalosporin and carbenicillin. We try to avoid using aminoglycosides in this situation because of the possible synergistic renal tubular damage between *cis*-platinum and aminoglycoside antibiotics. In our earlier patients some of the more significant transient nephrotoxic effects were seen in this clinical situation where aminoglycosides were used.

(*e*) Vigorous attention is paid to saline hydration, regardless of the patient's oral intake. All patients receive iv hydration (100 ml/hour of normal saline) for 12 hours prior to institution of the chemotherapy and for the entire 5-day course of *cis*-platinum.

Combination treatment with *cis*-platinum, vinblastine, and bleomycin (with or without Adriamycin) consistently produces a 60%–70% CR rate with a further 10%–15% of patients rendered disease-free following surgical removal of residual disease. Furthermore, chemotherapy can significantly alter the course of this disease, even when a CR is not seen, altering the histology to a more benign form (teratoma) and perhaps accounting for the prolonged PRs seen in several patients.

The results of *cis*-platinum combination chemotherapy regimens clearly indicate that disseminated testicular cancer is very responsive to chemotherapy, and that a high percentage of patients, even those with far-advanced disease, are potentially curable. The CR rate of 65%–70% and the overall disease-free status of 80% are as high as those seen in any adult malignancy treated with chemotherapy.

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Vinblastine, Bleomycin, and *cis*-Dichlorodiammineplatinum(II) in Disseminated Testicular Cancer: Preliminary Report of a Southwest Oncology Group Study [1]

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SUMMARY

Combination induction chemotherapy consisting of vinblastine, bleomycin, and *cis*-dichlorodiammineplatinum(II) was administered to 126 patients with advanced germ cell tumors of the testis. Following this 4-month induction therapy, maintenance treatment consisting of chlorambucil and actinomycin D alternating monthly with vinblastine was given for an additional 20-month period. Sixty-four patients (51%) achieved a complete remission, while 33 patients (26%) achieved a partial response. Responses were observed in all histologic categories. Seven patients (11%), who were in complete remission, have relapsed at a median time of 5 months. Median duration of remission is > 12 months for complete responders and 4 months for partial responders. Significant leukopenia was noted in 57% of evaluable courses and significant thrombocytopenia was noted in 18%. Less than 20% of patients were noted to have nephrotoxicity. Other toxic effects observed were gastrointestinal (42%), dermatologic (15%), pyrexia (14%), neuromuscular (9%), and pulmonary (< 5%). Several pretreatment parameters are currently being evaluated to determine the impact on complete remission rate and survival.

[Cancer Treat Rep 63:1663–1667, 1979]

Chemotherapy for testicular neoplasms of germ cell origin has had a long history of success from the standpoint of responsiveness to a wide spectrum of anticancer drugs (1–6). Since 1960, when Li et al (7) published the first account of combination chemotherapy in metastatic testicular cancer, numerous investigators have modified Li's original schedule and/or drug combination in an attempt to improve the results (8–10). Overall response rates in these studies ranged from 50% to 70%, which included 10%–30% complete remissions. Significantly, about one half of the complete responders are long-term survivors and represent potential chemotherapy "cures." Samuels (11) and Spigel and Coltman (12) have shown that the combination of vinblastine and bleomycin appears to be synergistic when the activity of each drug singly is compared to the activity of the combination.

cis-Dichlorodiammineplatinum(II) (*cis*-platinum) is one of a group of coordination compounds of platinum identified by Rosenberg et al (13) that strongly inhibits bacterial replication. Clinical trials with *cis*-platinum were initiated in 1971 under the auspices of the Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md). Cumulative results in advanced testicular cancer indicate an overall response rate of > 60%, making it the most active single agent in this disease category (14).

In April 1976, based on our phase I study (15) and the encouraging reports of Einhorn and Donohue (16) and

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Cvitkovic et al (17), the Southwest Oncology Group began a phase II study of vinblastine, bleomycin, and *cis*-platinum in advanced testicular cancer. This report constitutes our preliminary observations to date.

MATERIALS AND METHODS

One hundred and twenty-six patients with histologically proven testicular carcinoma of germ cell origin are the subjects of this report. All patients had clearly measurable metastatic disease and/or an elevated serum β subunit of human chorionic gonadotropin (HCG). Additional eligibility criteria included: no prior exposure to any of the chemotherapeutic agents used in this study, the absence of central nervous system metastases, an absolute granulocyte count of $> 1000/mm^3$ and a platelet count of $> 100{,}000/mm^3$, normal renal function defined as a BUN of ≤ 20 mg/100 ml, a serum creatinine of ≤ 1.2 mg/100 ml, and a creatinine clearance of ≥ 75 ml/minute with no evidence of obstructive uropathy, and adequate pulmonary function defined as a vital capacity or forced expiratory volume > 70% of the predicted value. Patients with pure seminoma were eligible for this study provided there was evidence of extranodal metastatic disease or documented failure of radiation therapy. Pretreatment evaluation included complete history and physical examination, performance status, hemogram, 17-channel screening profile, serum β subunit of HCG, chest roentgenogram with whole-lung tomograms, iv pyelogram, serum creatinine and creatinine clearance, audiogram, and pulmonary function tests. Brain, bone, and liver scans were performed when clinically warranted. During the study, hematologic and roentgenographic evaluation of each patient was performed at monthly intervals at least.

Drug Regimen

The treatment scheme is shown in table 1. Vinblastine, bleomycin (total dose, 200 units/m^2), and *cis*-platinum were administered over the initial induction period of 4 months. Patients in complete or partial remission received maintenance therapy consisting of chlorambucil and actinomycin D, alternating monthly with vinblastine, for an additional 20 months. Chemotherapy was discontinued after a total of 24 months and patients were followed until evidence of clinical relapse. Those patients who had received prior irradiation to > 25% of bone marrow-bearing areas were considered to have impaired marrow reserve and received a 25% reduction in the dose of vinblastine. Each treatment course was administered provided there was sufficient evidence of recovery from the nadir wbc and platelet counts observed with the previous course of therapy. *cis*-Platinum was withheld if there was evidence of nephrotoxicity (creatinine of > 1.3 mg/100 ml and BUN of > 30 mg/100 ml); it was reinstituted once normalization occurred. Bleomycin was permanently discontinued if pulmonary toxicity developed, manifested either by pulmonary infiltrate or a decrease of ≥ 25% in baseline pulmonary function.

TABLE 1.—Treatment scheme

Drug	Dose	Treatment (Days)
	Induction therapy (4 mos)	
Vinblastine	12 mg/m^2	1, 29, etc
Bleomycin	15 units/m^2	Biweekly until total dose of 200 units/m^2 is reached
cis-Platinum	15 mg/m^2	1–5, 29–33, etc
	Maintenance therapy (20 mos)	
Actinomycin D	1.5 mg/m^2	1, 58, etc
Chlorambucil	10 mg/m^2 orally	4–8, 61–66, etc
Vinblastine	12 mg/m^2	29, 87, etc

Response Criteria

Responses were classified as follows: *complete remission*—complete disappearance of all measurable disease and symptoms for a minimum of 4 weeks and normalization of HCG, if initially evaluated; *partial remission*—> 50% but < 100% reduction in the sum of the areas of all measurable lesions, including HCG, for a minimum of 4 weeks; *progression*—> 50% increase in the sum of the areas of all measurable lesions and/or the appearance of new lesions; *relapse*—appearance of new lesions or the reappearance of old lesions in patients who had achieved complete remission, or an increase of ≥ 50% in the sum of the products of the diameters of the measured tumors over that which was obtained at the time of maximum regression for patients who had achieved partial remission.

Patients

All 126 patients had a median age of 27 years (mean, 29; range, 15–61), with a median Karnofsky performance status of 90 (mean, 82; range, 20–90). For all patients, diseases were categorized histologically by the World Health Organization classification which is defined as follows: *tumors showing a single cell type*—seminoma, embryonal carcinoma, yolk sac tumor, teratoma, and choriocarcinoma; and *tumors showing more than one histologic pattern*—embryonal plus teratoma with or without seminoma, embryonal carcinoma plus seminoma, teratoma plus seminoma, and any combination with choriocarcinoma. Table 2 describes the nature of prior treatment as well as sites of metastatic disease in this treatment population.

TABLE 2.—Patients

	No. of patients
Prior treatment	
Orchiectomy alone	77
Orchiectomy + lymphadenectomy	23
Orchiectomy + lymphadenectomy + radiation therapy	10
Orchiectomy + radiation therapy	16
Metastatic sites	
Lung (including mediastinum and hilar lymph nodes)	81
Abdominal and/or retroperitoneum	55
Lymph nodes, peripheral	23
Liver	13
Other	14

RESULTS

Response to Therapy

All patients entered in this study who received at least 1 day of therapy were considered evaluable for response. Of these 126 patients, there were 64 complete remissions (51%) and 33 partial remissions (26%), for an overall response rate of 77% (table 3). Seven patients (11%) who were in complete remission have relapsed at a median time of 5 months (range, 3–7) after documentation of that response. Median duration of remission is > 12 months (range, 1+ to 24+) for complete responders and 4 months (range, 1+ to 10+) for partial responders. Twelve patients (19%) who have complete remission have been in remission for > 1 year.

Figure 1 shows the remission duration for patients in complete and partial remission. Remission duration is significantly longer for patients in complete remission than for patients in partial remission ($P = 0.002$). Relapse-free rates for patients in complete remission are 87% at 6 months and 82% at 12 months, while the relapse-free rate for patients in partial remission is only 46% at 6 months.

Hematologic Toxicity

Table 4 shows the hematologic toxicity that was observed through the four courses of induction therapy for all evaluable patients. There was no evidence to suggest cumulative bone marrow toxicity with succeeding courses of treatment. Significant leukopenia (< 3000 cells/mm^3) was noted in 57% of evaluable courses and significant thrombocytopenia (< 100,000 cells/mm^3) was noted in 18%. No appreciable hematologic toxicity was observed during maintenance therapy. One patient died of pneumonia with a wbc count of 500/mm^3; this was considered a toxic drug death.

Nonhematologic Toxicity

Nausea and vomiting were reported in 42% of patients, while alopecia was noted in 17%, dermatologic toxicity was noted in 15%, fever was noted in 14%, and neuromuscular toxicity was noted in 9%. The frequency of altered pulmonary function in the study did not exceed 5%; however, one patient developed progressive pulmonary insufficiency secondary to bilateral pulmonary fibrosis after receiving a total dose of bleomycin of 200 units/m^2.

In 16% of the patients, the BUN was noted to rise above 20 mg/100 ml, while in 7% of the patients, the serum creatinine rose above 2.0 mg/100 ml. In only one

TABLE 3.—Response rates

World Health Organization classification	No. of— Patients	Complete remissions	Partial remissions	Relapses after complete remissions
Seminoma	8	2 (25)*	1 (13)	1 (13)
Embryonal carcinoma	52	37 (71)	7 (13)	4 (8)
Teratoma	5	2 (40)	1 (20)	0
Choriocarcinoma	4	1 (25)	1 (25)	0
Teratocarcinoma	26	10 (38)	9 (35)	1 (4)
Embryonal carcinoma + any other type	8	4 (50)	4 (50)	0
Teratoma + seminoma	3	1 (33)	0	0
Choriocarcinoma + any other type	20	7 (35)	10 (50)	1 (5)
Totals	126	64 (51)	33 (26)	7 (11)

*Nos. in parentheses = % of total No. of patients.

TABLE 4.—Hematologic toxicity

Course No.	No. of evaluable patients	Mean wbc nadir (cells × $10^3/mm^3$)	Median wbc nadir (cells × $10^3/mm^3$)	Range	No. of evaluable patients	Mean platelet nadir (cells × $10^3/mm^3$)	Median platelet nadir (cells × $10^3/mm^3$)	Range
1	102	3.3	3.2	0.1–11.6	96	192	171	30–435
2	91	2.6	2.4	0.3–6.3	88	166	160	9–457
3	64	3.1	2.7	0.8–10.1	58	163	154	35–360
4	42	3.0	2.7	0.9–9.0	41	143	131	23–387

patient did the serum creatinine exceed 4 mg/100 ml. In this study, serial creatinine clearances were not obtained with any regularity and the long-term effects of *cis*-platinum on renal function are not yet known. Tinnitus was noted in only four patients. Clinically appreciable hearing loss was not noted, however.

DISCUSSION

Historically, germ cell tumors of testicular origin have been noted to be responsive to a wide spectrum of chemotherapeutic agents. However, to achieve any meaningful impact on survival, a complete response must be achieved since partial or mixed responses have little impact on duration of survival. Furthermore, the achievement of a complete response appears to offer the patient a chance for long-term survival and possible "cure."

Prior to the introduction of *cis*-platinum into the treatment of testicular cancer, most large series rarely reported complete response rates of > 30%. Our study, as well as others which included *cis*-platinum, have resulted in complete remission rates of > 50% with a < 25% relapse rate. The recent reports of Willis and Haj Du (18) and Kong et al (19), who found mature teratoma in some patients who had not reached an apparent complete response, suggest that an even higher percentage of patients may achieve a disease-free status with surgical intervention.

We are currently evaluating what impact, if any, age, performance status, sites of metastases, pretreatment LDH and β subunit of HCG levels, histology, pretreatment bone marrow status, pretreatment surgery, and prior radiation therapy have on complete remission rates and survival.

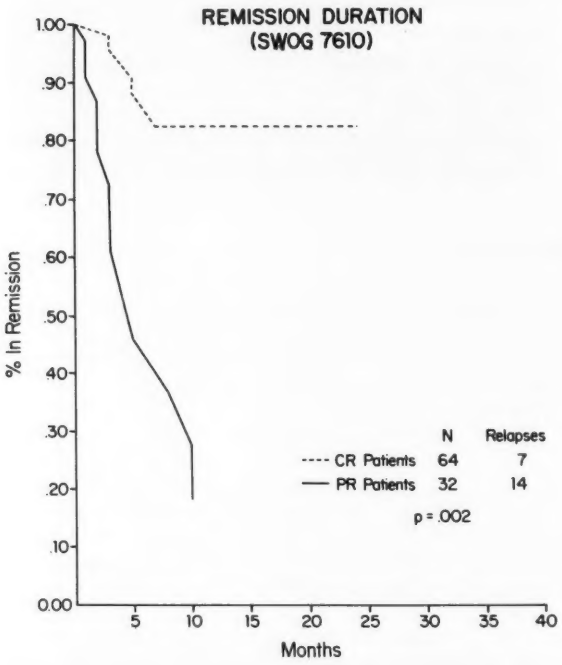

FIGURE 1.—Remission duration for complete and partial responders.

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Treatment of Metastatic Nonseminomatous Testicular Cancer: A Preliminary Report of Induction Chemotherapy Followed by Maintenance Chemotherapy or Radiotherapy[1]

Roland W. Sonntag,* Hans Jörg Senn, and Franco Cavalli[2]

SUMMARY

Patients with metastatic nonseminomatous testicular cancer received an induction regimen consisting of bleomycin in 24-hour infusions and bolus iv doses of vinblastine followed by an Adriamycin and *cis*-dichlorodiammineplatinum(II) combination. Patients achieving complete remission after one or two cycles of this induction chemotherapy were then randomized to receive either radiotherapy (RT) to the previously involved tumor areas or maintenance chemotherapy (MCT) with CCNU, methotrexate, and vinblastine for 2 years. Among 62 evaluable patients, induction chemotherapy achieved 15 (24%) partial remissions and 35 (56%) complete remissions. Two patients with partial remission and single pulmonary metastases were rendered disease-free by surgical resection of residual tumor. Twenty patients received MCT and 15 received RT. To date, median survival is 10.8+ months in the MCT group with five relapses and 12.5 months in the RT group with two relapses. Toxicity in the induction phase was moderately severe with two drug-related deaths.

[Cancer Treat Rep 63:1669–1674, 1979]

Although significant advances in the treatment of nonseminomatous testicular carcinoma have been made during the past 18 years, the concept of possible curative management in the far-advanced metastatic stage of the disease is relatively new. As treatment improved through the availability of new and effective drugs, especially vinblastine (VBL) (1) and bleomycin (BLM) (2), and the use of these agents in combination (3,4), remission rates of 60%–80% were no longer uncommon. It also became obvious that only complete remissions (CRs) were of benefit to the patient in terms of remission duration and survival (3,5,6), with a probability of cure approaching 50% (1,6).

In May 1976 the Swiss Group for Clinical Cancer Research (SAKK) initiated a study with two primary goals. The first was to achieve a high CR rate with an intensive polychemotherapy induction phase of treatment. A four-drug sequential program using two two-drug combinations was developed to enable higher dosage of the individual agents than would be possible if all four were given simultaneously. All four drugs had been of proven value in treating testicular carcinoma (1,2,7,8). The second goal was to determine the relative values of maintenance chemotherapy (MCT) versus radiation therapy (RT) for consolidation of these CRs.

MATERIALS AND METHODS

All patients with histologically proven testicular carcinoma and radiologic or clinical evidence of inoperable metastases beyond the inguinal lymph nodes were eligible for the trial. Patients with pure seminomas were ineligible. Previous therapy could not have included any of the drugs used in the induction phase of treatment. Patients having previously received other drugs or RT were not excluded, provided that pre-existing therapy-induced toxicity had fully subsided.

In addition to a complete physical examination, the following parameters were recorded at entry in the study:

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hemoglobin, platelet, leukocyte, and differential blood cell counts, BUN or serum creatinine, alkaline phosphatase and SGOT, α-fetoprotein and serum or urine human chorionic gonadotropin, and chest roentgenogram. Liver scans or abdominal lymphangiograms were performed whenever indicated. All measurable tumor lesions were documented. The regular control of these parameters was defined in the protocol.

The induction program consisted of two sequential combination regimens (fig 1). The *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) dose was followed by forced diuresis (2000 ml of glucose and saline infusion containing 40 g of mannitol and 40 mg of furosemide given over 6–8 hours). The Adriamycin (ADM) and *cis*-platinum doses were each increased to 60 mg/m^2 after the first 21 evaluable patients showed only minimal hematologic toxicity and virtually no nephrotoxicity with a 50-mg/m^2 dose.

Patients achieving CR after the 12-week induction treatment were then randomized to receive either MCT for 2 years or RT to the areas of previous tumor involvement. If a partial remission (PR) was attained in patients with solitary pulmonary metastases, surgical resections of residual tumor were performed followed by MCT. In the other patients with PR, the above induction chemotherapy was repeated and patients with CR after this second induction treatment were then randomized to receive either MCT or RT whereas patients with persisting PRs were given MCT. Patients with a < 50% response, no change, or progressive disease were dropped from the study as treatment failures.

MCT consisted of CCNU (100 mg/m^2) every 8 weeks plus alternating weekly VBL (7 mg/m^2) and oral methotrexate (20 mg/m^2) (fig 1).

CR was defined as complete disappearance of all measurable and nonmeasurable tumor parameters as well as a return to normal of tumor markers such as α-fetoprotein and human chorionic gonadotropin if these were initially elevated. A PR indicated a reduction of > 50% in the product of two perpendicular tumor diameters.

Initial drug doses were reduced by 25% if the patient had undergone extensive RT, especially to the abdominal and pelvic regions, within 2 months of starting treatment. If no undue toxicity was observed during the first cycle of BLM and VBL, the doses were increased to 100%. Normal renal function (with a BUN level < 30 mg/dl or a serum creatinine level < 1.8 mg/dl) was mandatory for BLM or *cis*-platinum administration. The doses of subsequent courses of treatment were reduced by 25% for the following reasons: persisting leukopenia (between 3000 and 4000 cells/mm^3) or thrombocytopenia (between 90,000 and 120,000 cells/mm^3), a leukocyte nadir < 1000/mm^3 or a platelet nadir < 20,000/mm^3, persisting mucositis, severe obstipation with symptoms of ileus, or fever > 39°C not controlled with prednisone. MCT doses were reduced by 50% for leukocyte counts between 2500 and 4000/mm^3 or platelet counts between 50,000 and 100,000/mm^3. Therapy was withheld if lower values or persisting mucositis occurred.

Megavoltage RT was applied to all known areas of tumor involvement at the start of the study. The fields were to extend 4 cm beyond the known tumor area wherever possible. A dose of 4000 rads given over 4–5 weeks for field areas < 300 cm^2 was planned. For field areas

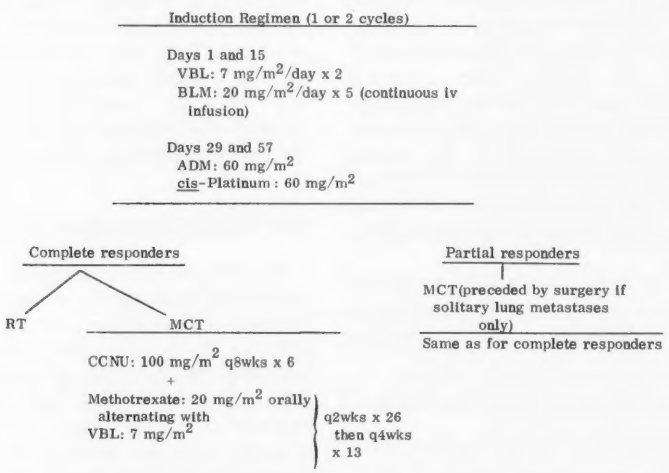
Induction Regimen (1 or 2 cycles)
Days 1 and 15
VBL: 7 mg/m2/day x 2
BLM: 20 mg/m2/day x 5 (continuous iv infusion)
Days 29 and 57
ADM: 60 mg/m2
cis-Platinum : 60 mg/m2
Complete responders
RT
MCT
CCNU: 100 mg/m2 q8wks x 6
+
Methotrexate: 20 mg/m2 orally alternating with VBL: 7 mg/m2
q2wks x 26 then q4wks x 13
Partial responders
MCT(preceded by surgery if solitary lung metastases only)
Same as for complete responders

FIGURE 1.—Induction and maintenance schedules for metastatic testicular carcinoma.

> 300 cm^2 the dose was reduced to 3000 rads. The total dose to the kidneys was not to exceed 1700 rads over 4 weeks and the total dose to the liver was limited to 2500 rads. If the irradiated lung volume was ≤ 30% of the total lung volume, a midline dose of 2500 rads was given with daily doses of 180–200 rads. For irradiated volumes up to 50% of the total lung volume the dose was 1800 rads with daily doses of 150–160 rads. For irradiated volumes > 50% of the total lung volume the dose was 1500 rads with 100-rad daily doses.

RESULTS

As of July 1978, 82 patients were entered in the study. Of these, 20 were not evaluable. Ten had not yet completed their first induction cycle. Gross protocol violations with regard to treatment sequence, timing, or randomization occurred in eight. One patient was ineligible because no measurable tumor was present and another developed an acute glomerulonephritis so that *cis*-platinum could not be given.

Of the 62 evaluable patients, there were 15 (24.2%) patients with PRs and 13 (87%) of these relapsed. The median duration of PR was only 3.1 months (range, 0.3–8.3 months). The median survival was 6.8 months (range, 0.8–22 months). The longest survivor with PR attained a good PR from initial treatment with BLM and VBL but was removed from the study when progression occurred during the ADM/*cis*-platinum phase of treatment. The remaining lung tumor was removed surgically. This was followed by four BLM/VBL courses.

There were 37 (59.7%) CRs; two of these resulted from resection of a residual lung lesion. Only nine (24.3%) of the patients with CR relapsed including the two patients who had undergone surgical resection of residual lung metastases. Both of these tumors contained choriocarcinoma components and one patient refused MCT.

Twenty-nine patients achieved CR with one induction treatment (22 with BLM/VBL only) and six after a second induction treatment. The actual tumor effect of ADM/*cis*-platinum in this study is very difficult to evaluate. Only 39 patients had measurable disease that could be evaluated during the ADM/*cis*-platinum phase of treatment. Twenty-six of these showed further tumor regression with ADM/*cis*-platinum.

After randomization, 20 patients with CR received MCT and 15 with CR were irradiated. Five relapses (25%) have occurred in the MCT group between 2.1 and 13.1 months. Two of these patients are still alive. The median remission duration for the MCT group is 7.75+ months (range, 0–23 months) and the median survival, with 17 patients still alive, is 10.8+ months (range, 3.7–25.3 months).

In the RT group, there have been only two (13%) relapses at 4.8 and 6.7 months. One of these patients is still alive. This gives a median remission duration of 8.3+ months (range, 0–25.8 months) and a median survival of 12.5+ months (range, 3–27.7 months) for this group.

Toxicity encountered during the induction chemotherapy phase of treatment was moderately severe. It manifested itself mainly in the form of hematologic toxicity following the BLM/VBL cycles and was less severe with ADM/*cis*-platinum (table 1).

Other forms of toxicity included BLM-induced fever. When this occurred, patients were given prednisone during subsequent BLM administration. Therefore, only 70 initial BLM/VBL courses are evaluable in this regard and they resulted in 55 febrile episodes; fever > 39°C was seen in 21 patients. A complete or almost complete alopecia was noted after the first BLM/VBL therapy in 55 patients. This was seen in another 11 patients following ADM/*cis*-platinum. Obstipation with abdominal pain and symptoms of ileus resulting from BLM/VBL treatment was encountered in 46 of the 89 courses and was considered moderately severe in 20. Mucositis was frequent with BLM/VBL (39 of 89 courses) and was moderately to very severe in 18 cases. Stomatitis was seen only five times with ADM/*cis*-platinum. Nausea and vomiting was

TABLE 1.—Hematologic toxicity during induction phase

	No. of courses				
	Leukocyte count (cells × $10^3/mm^3$)		Platelet count (cells × $10^3/mm^3$)	Hemoglobin level	
	1.0–1.9	< 1.0	< 50.0	3–4 g/100 ml	> 4 g/100 ml
BLM/VBL (total = 89 courses)	29	35	18	17	10
ADM/*cis*-platinum (total = 82 courses)	14	3	2		
MCT (total = 24 courses)	14	0	1	NE*	NE

*NE = not evaluated.

recorded in 38 of 89 BLM/VBL courses and was moderate to severe in 24. This form of toxicity was observed in 71 of 82 courses of ADM/*cis*-platinum and was judged to be moderate to severe in 61.

Renal toxicity with *cis*-platinum was not a problem with the modes of administration used in this study. Elevated BUN and/or serum creatinine values were seen in six of 64 evaluable courses. Only two patients had persisting serum creatinine values between 1.7 and 2.0 mg/dl. Myalgia and arthralgia were common with BLM/VBL. Only one case of BLM-induced lung toxicity has been documented. It was not fatal.

Two drug-related deaths occurred during induction treatment. The first patient, with extensive abdominal tumor masses, underwent laparotomy on Day 9 of treatment for intestinal obstruction. Three days after surgery the peripheral granulocyte count was 36/mm^3. By Day 16 the leukocyte count had recovered to 9900/mm^3 and, in violation of the protocol, another full dose of BLM/VBL was given. This resulted in wound dehiscence, fecal peritonitis, agranulocytosis, sepsis, and death on Day 25. The second death occurred in a patient with initially normal serum creatinine values (0.7–1.2 mg/dl). Again, despite a leukocyte nadir of 500/mm^3 during the first BLM/VBL course, full doses of these drugs were repeated. On Day 3 of this second course the serum creatinine value was 2.0 mg/dl and continuously rose to 9.5 mg/dl. The leukocyte count subsequently fell to 90/mm^3 with 0 granulocytes, and the patient died on Day 26 of the study with an *Escherichia coli* sepsis. The cause of the renal insufficiency which was present before the patient became septic and certainly contributed significantly to the fatal outcome is not entirely clear. It may have resulted from a 5-day gentamicin treatment (240 mg given daily on Days 9–13).

DISCUSSION

The first goal of this study, to attain a high CR rate with induction chemotherapy, has been achieved (59.7%). Even if the five patients with short CR durations of < 8 months (including two patients with resection of residual pulmonary disease and two with poorly evaluable abdominal disease) are excluded as false interpretations of disease status, the CR rate remains 52%. Also disregarded in these results are at least two CRs in the nonevaluable group. One had to be dropped from the study because of an acute glomerulonephritis which made *cis*-platinum treatment impossible. Another never received RT as per randomization. On the other hand, two patients with $> 50\%$ PRs, one who died of a massive pulmonary embolus after the second BLM/VBL course and one who died of pulmonary hemorrhage into the tumor bed before a second induction could be given, are included in these figures.

The two toxic deaths occurred in the face of frank pro-

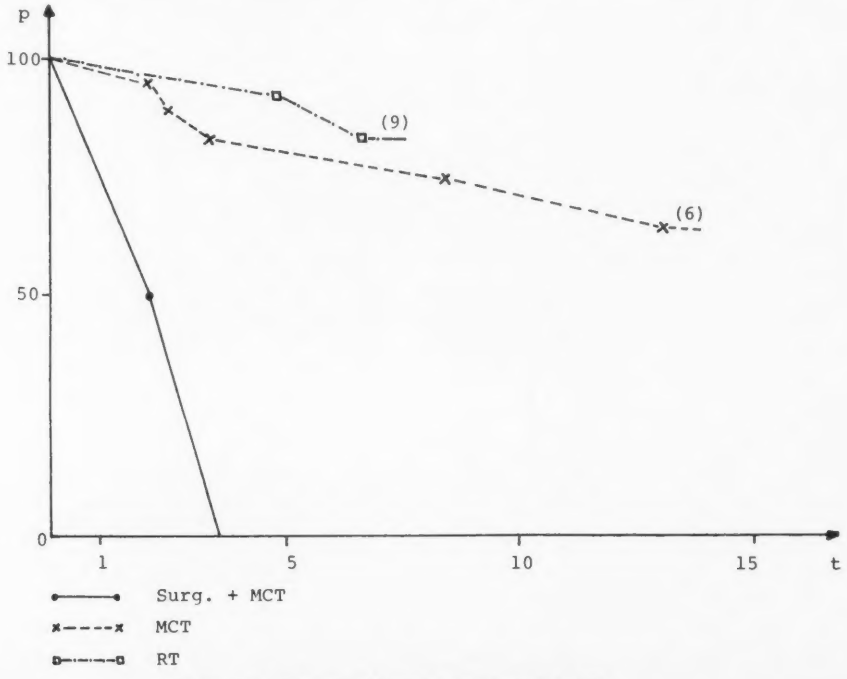

FIGURE 2.—Remission duration (patients with CR).

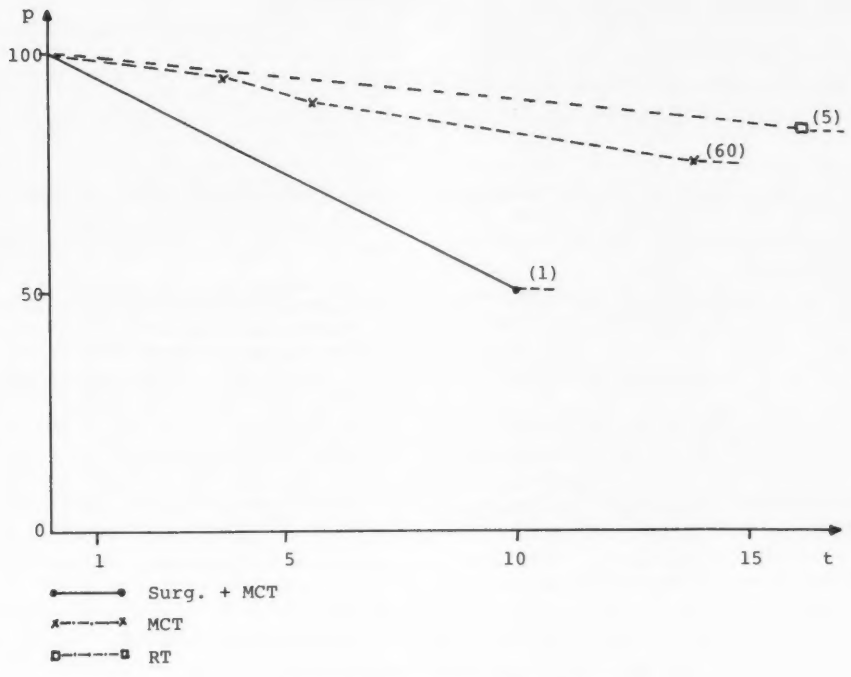

FIGURE 3.—Survival (patients with CR).

tocol violations. They may possibly have been avoided by strict adherence to the protocol stipulations of dose reduction. This lethal toxicity rate of 3.2% with a minimal CR rate of 52% without surgery compares well to the 6.4% (three of 47 patients) lethal toxicity rate reported in the only other study of this size to date with a CR rate > 50% with chemotherapy alone (9).

Unexpected toxicity in the RT arm of this study was not encountered despite the intensive chemotherapy pretreatment. Even six total lung irradiations following BLM doses of up to 700 mg (270–700 mg) have been tolerated to date. The two relapses after RT have occurred in the lung. One was a relapse outside single bilateral radiation fields; the other occurred following total-lung irradiation. It appears, therefore, that RT is of definite value for the consolidation of CRs induced by chemotherapy in this disease. Whether it is superior to MCT still remains to be seen.

Failure to achieve long-term remission in our two patients undergoing surgical resection of residual pulmonary disease is disappointing. It does not correlate with our own previous experience or that of Einhorn and Donohue (9). One patient with a survival of only 10 months had refused MCT; the other is alive with disease at 16 months. Surgery was very likely performed too early in these patients and should have been preceded by a second course of induction therapy.

A comparison of the patient characteristics in the RT- and MCT-treated groups (table 2) shows the RT patients to be slightly younger. Twenty percent of their tumors

TABLE 2.—Patient characteristics

		Histology		Localization	
	Median age (yrs)	Embryonal cell	Chorio-carcinoma	Thorax	Abdomen ± thorax
All patients	28	40%	14.5%	53.2%	43.5%
Complete responders	29	60%	11.4%	51.4%	42.9%
Complete responders: MCT	28.5	60%	5%	55.0%	40.0%
Complete responders: RT	24	60%	20%	46.7%	46.6%

contained choriocarcinomatous components, and there was abdominal tumor present in almost 50%. Table 2 also suggests that patients with tumors of the embryonal cell type, with or without seminoma, but lacking other histologic tissue types were more likely to undergo CR with induction chemotherapy than patients with other forms or mixed tumors. There were 40% such "pure" embryonal cell tumors in the entire patient population, 60% in those attaining CR, and only four (16%) in the 25 patients not attaining CR. This last difference for the nonresponders is statistically significant ($P = 0.003$).

A definite evaluation of remission duration and survival is not yet possible since 28 of the 35 patients in CR remain tumor-free (0–25.8+ months) and 31 are still living 3+ to 27.7+ months since initiation of treatment (figs 2 and 3).

In view of the results achieved, we feel that the toxicity encountered with this treatment regimen is acceptable. Lethal toxicity may well be avoidable if strict precautions for dose adjustment and patient and laboratory controls are followed. The risk and discomfort as well as the expenditure of time and effort seem to be adequately compensated for in terms of survival and possible cure in this group of young patients.

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Chemotherapy for Advanced Germinal Cell Neoplasms: Preliminary Report of an Eastern Cooperative Oncology Group Study

William D. DeWys,* Colin Begg, Robert Slayton, Richard G. Hahn, and Isadore Brodsky [1,2]

SUMMARY

A series of 45 evaluable patients with stage III germ cell tumors have been treated between July 1977 and May 1978, with two cycles of an intensive five-drug regimen including cyclophosphamide, actinomycin D, vinblastine, bleomycin, and *cis*-dichlorodiammineplatinum(II) (*cis*-platinum). The patients were randomly assigned to receive the *cis*-platinum as a high-dose 1-hour infusion with mannitol diuresis or as a low-dose 8-hour infusion without diuresis. The overall response rates were 36% complete response (CR) and 47% partial response (PR) after two cycles of induction therapy. Patients who achieved a CR or PR after two cycles were randomized to continue treatment with either a three-drug program of vinblastine, actinomycin D, and *cis*-platinum or a cyclic crossover regimen of three regimens (each containing three drugs) given in sequence at 3-week intervals with the entire sequence repeated at 9 weeks. During continuing treatment a minimum of five patients improved from PR to CR, so that the minimum CR rate will be 47%. A major determinant of response was extent of disease, with limited-disease patients having an 83% CR rate and advanced-disease patients having a 22% CR rate. At present, there are no statistically significant differences between response rates for the two induction regimens. Patients have not been followed long enough on the continuing treatment arms to allow evaluation of improvement from PR to CR, duration of response, or duration of survival. Hematologic and renal toxicity were not significantly different on the two induction treatment arms.

[Cancer Treat Rep 63:1675–1680, 1979]

In July 1977, the Eastern Cooperative Oncology Group began a protocol for treatment of stage III testicular tumors; in May 1978, the protocol was expanded to include extratesticular germ cell tumors. The choice of drugs in this protocol was based on the favorable results reported by the Memorial Hospital (New York, NY) group for a five-drug regimen (1). A major difference from the Memorial Hospital protocol was the inclusion of *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) in each cycle of treatment rather than every third cycle. For the first two cycles patients were randomly assigned to receive the *cis*-platinum at a high dose with mannitol di-

[1]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone, Chairman*, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare. The following institutions and investigators participated in the study: Northwestern University Medical Center, Chicago, Ill (*W. D. DeWys*, CA-17145); Sidney Farber Cancer Institute, Boston, Mass (*C. Begg*, CA-23318); Rush-Presbyterian-St. Luke's Medical Center, Chicago, Ill (*R. Slayton*, CA-10948); Mayo Clinic and Mayo Foundation, Rochester, Minn (*R. G. Hahn*, CA-13650); and Hahnemann Medical College, Philadelphia, Pa (*I. Brodsky*, CA-13611). Other participating institutions were: Albany Medical College (CA-06594); Albert Einstein College of Medicine (CA-14958); American Oncologic Hospital (CA-18281); Baystate Medical Center (CA-20182); Boston University Medical Center (CA-11109); Harbor General Hospital (CA-21091); Johns Hopkins Oncology Center (CA-16116); National Cancer Institute; New York University Medical Center (CA 16395); University of Ottawa, Ontario, Canada; Hospital of the University of Pennsylvania (CA-15488); University of Montreal Affiliated Hospitals, Quebec, Canada (CA-21653); University of Rochester Cancer Center (CA-11083); University of Pretoria, South Africa (CA-21692); and Yale School of Medicine (CA-06510).

[2]We wish to thank Nirsa Negron for her secretarial assistance.

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uresis as described by Cvitkovic et al (1) or as an 8-hour infusion as described by Merrin (2). He had observed five complete responses (CR) and three partial responses (PR) in nine testicular cancer patients treated with *cis*-platinum in an 8-hour infusion.

A second goal of the protocol was a comparison of two continuing treatment programs aimed at maximizing the durations of response and survival. A three-drug regimen was compared with a cyclic crossover regimen in which a total of six different drugs were arranged into three triplets given at 3-week intervals with the entire sequence repeated at 9 weeks. All six drugs had known activity against testicular carcinoma (3–11).

This paper will be a preliminary report of the results of this protocol which is still in progress. Data will be coded because differences have not reached statistical significance and the protocol continues to accrue patients.

MATERIALS AND METHODS

Sixty-three male patients with a histologically confirmed germ cell neoplasm which was stage III or recurrent after primary surgery or radiotherapy were entered in this protocol between July 21, 1977, and May 30, 1978. One patient has been excluded from this analysis because of uncertainty about the histologic diagnosis. For 17 patients data submission is not complete enough for analysis, leaving 45 patients for inclusion in this report. All patients had a performance status of ≥ 3 using the Eastern Cooperative Oncology Group definitions and all gave written informed consent. All patients had measurable or evaluable disease. Measurable disease included well-defined metastatic nodules and liver involvement if at least two measurements below the costal margin (right, xiphoid, left) were > 5 cm. Evaluable, nonmeasurable disease included lymphangitic or confluent multinodular lung metastases, ascites, or pleural effusions caused by metastases and not responsive to diuretics. Patients were excluded if they had pure seminoma, did not have adequate hepatic, renal, and bone marrow functions (bilirubin < 1.5 mg/100 ml, SGOT < 60 IU/ml, BUN < 25 mg/100 ml, creatinine < 1.5 mg/100 ml, wbc count > 4000/mm^3, and platelet count > 100,000/mm^3), had had prior chemotherapy, or had an acute intercurrent complication.

Patients were stratified as to performance status (0–1 versus 2–3) and histologic type and were randomly assigned by a central randomization office to receive one of two induction regimens shown in figure 1. After two cycles, patients who had achieved a CR (complete disappearance of all clinically detectable malignant disease and normal serum markers) or PR (> 50% reduction of the sum of the products of perpendicular tumor diameters, > 30% reduction of the sum of liver measurements below the costal margin with improved liver function, or > 50% regression of evaluable nonmeasurable disease confirmed by two observers independently) were randomly assigned to continue treatment (fig 2) while nonresponders were removed from the study.

After the initial treatment, subsequent doses were adjusted based on toxicity at the time of the next scheduled treatment and worst toxicity occurring between treatments (table 1). If at the time of scheduled treatment the wbc count was > 4000/mm^3 and the platelet count was > 100,000/mm^3, the usual doses of chemotherapy were given. If the wbc count was 3000–4000/mm^3 or if the platelet count was 75,000–100,000/mm^3, 50% doses were given,

Treatment A: modified VAB III (high-dose *cis*-platinum), 2 cycles
- Cyclophosphamide, 600 mg/m^2 iv on Day 1
- Actinomycin D, 1.0 mg/m^2 iv on Day 1
- Vinblastine, 4 mg/m^2 iv on Day 1
- Bleomycin, 20 units/m^2/24 hrs continuous iv infusion on Days 1–7
- *cis*-Platinum, 120 mg/m^2 over 1 hr iv with a mannitol-induced diuresis (do not reduce dose for hematologic toxicity) on Day 8

Repeat cycle q21 days.

Treatment B: modified VAB III (low-dose *cis*-platinum), 2 cycles
- Cyclophosphamide, 600 mg/m^2 iv on Day 1
- Actinomycin D, 1.0 mg/m^2 iv on Day 1
- Vinblastine, 4 mg/m^2 iv on Day 1
- Bleomycin, 20 units/m^2/24 hrs continuous iv infusion on Days 1–7
- *cis*-Platinum, 40 mg/m^2 on Days 1 and 8 as an 8-hr infusion (does not require mannitol diuresis; do not reduce dose for hematologic toxicity)

Repeat cycle q21 days.

FIGURE 1.—Induction regimens.

VAP regimen
- Vinblastine, 4.0 mg/m^2 iv on Day 1
- Actinomycin D, 1.0 mg/m^2 iv on Day 1
- *cis*-Platinum, 40 mg/m^2 iv as a 1-hr infusion on Day 8

Cyclic crossover regimen

The following 3 regimens are to be used in sequence at 3-wk intervals with the entire sequence to be repeated at 9 wks:

AVP
- Actinomycin D, 0.4 mg/m^2 iv on Days 1–5
- Vincristine, 1.0 mg/m^2 iv on Days 1 and 8 (maximum single dose must be ≤ 2.0 mg)
- *cis*-Platinum, 40 mg/m^2 iv as a 1-hr infusion on Day 8

CAP
- Cyclophosphamide, 500 mg/m^2 iv on Day 1
- Adriamycin, 50 mg/m^2 iv on Day 1
- *cis*-Platinum, 40 mg/m^2 iv over 1 hr on Day 8

Adriamycin should be discontinued when the total cumulative dose reaches 450 mg/m^2 and thereafter this arm of the protocol will continue with cyclophosphamide and *cis*-platinum only

VAP
- Vinblastine, 4.0 mg/m^2 iv on Day 1
- Actinomycin D, 1.0 mg/m^2 iv on Day 1
- *cis*-Platinum, 40 mg/m^2 iv over 1 hr on Day 8

FIGURE 2.—Continuing treatment regimens.

TABLE 1.—Dose modifications

Toxicity	Drugs modified	Basis for modification	Dose given
Hematologic	Vinblastine, actinomycin D, cyclophosphamide, Adriamycin	1. Toxicity at time of scheduled treatment:	
		Wbc count 3000–4000/mm³	50%
		Wbc count < 3000/mm³	0
		Platelet count 75,000–100,000/mm³	50%
		Platelet count < 75,000/mm³	0
		2. Toxicity at nadir:	
		Wbc count < 1000/mm³	80%
		Platelet count < 25,000/mm³	80%
Renal	*cis*-Platinum	BUN > 25 mg/100 ml, creatinine > 1.5 mg/100 ml	Delay until recovered, then 75%
Mucosal	Vinblastine, actinomycin D, Adriamycin, bleomycin	1. Toxicity at time of scheduled treatment	Delay until clear
		2. Worst degree of toxicity > 2+	75%
Skin	Bleomycin	Discomfort	Delay until recovered
Pulmonary	Bleomycin	Abnormal physical examination, chest roentgenogram, or function test	0

and if the wbc count was < 3000/mm³ or if the platelet count was < 75,000/mm³, chemotherapy was delayed. If the nadir wbc count was < 1000/mm³ or if the nadir platelet count was < 25,000/mm³, doses of vinblastine, actinomycin D, cyclophosphamide, and Adriamycin were reduced by 20%. Drug doses were also adjusted for renal, mucosal, skin, and pulmonary toxicity as shown in table 1.

Patients had an initial examination including history and physical examination, tumor measurements, chest roentgenogram, iv pyelogram, cbc, chemistry profile, pulmonary function tests, β subunit of human chorionic gonadotropin, alpha-fetoprotein, and electrocardiogram. Blood cell counts were repeated weekly and tumor measurements, chest roentgenogram, chemistry profile, and serum markers were repeated at least every 3 weeks. All pathology material is being reviewed by a pathology panel headed by Dr. Phillip Gordon. Dr. F. K. Mostofi serves as a referee. Extent of disease was classified in accordance with several modifications of the classification system described by Samuels et al (12) as follows:

III A—Extension beyond diaphragm but still confined to the lymphatic system, ie, supraclavicular nodes.

III B1—Elevation of human chorionic gonadotropin or alpha-fetoprotein. No detectable gross tumor.

III B2—Minimal pulmonary disease. Up to five metastatic masses in each lung, with the largest diameter of any single lesion ≤ 2.0 cm.

III B3—Advanced pulmonary disease. Any mediastinal or hilar mass, neoplastic pleural effusion, or intrapulmonary mass > 2.0 cm.

III B4—Minimal abdominal disease. Minimal pulmonary disease plus positive lymphangiogram or positive computerized tomographic scan of abdomen with no ureteral displacement or palpable abdominal mass.

III B5—Advanced abdominal or visceral disease. Any palpable mass, ureteral displacement by enlarged periaortic nodes, or obstructive uropathy. Visceral disease (excluding lung) such as liver, gastrointestinal tract, and brain.

RESULTS

The CR rates were 35% and 36% respectively for treatments X and Y[3] after two cycles of treatment (6–8 weeks). At least five additional patients achieved a CR during continuing treatment, giving minimum CR rates of 52% and 41% respectively for treatments X and Y. The total response rates (CR plus PR) were 82% and 86% for treatments X and Y. An analysis of CR rate by histologic type is shown in table 2. No significant differences emerge for the two treatments. The highest response rate observed is for embryonal carcinoma (62% CR) and the lowest response rate observed is for choriocarcinoma (29% CR).

An analysis of CR rate by extent of disease for the two treatments is presented in table 3. The highest response rates were noted in patients who had positive markers only, supraclavicular nodes, minimal pulmonary disease, or minimal abdominal disease. The combined response for these groups was 15 CRs among 18 patients treated (83% CR). Low response rates were observed in patients with either advanced pulmonary or abdominal disease;

[3]"X" and "Y" are coded designations which refer to either treatment A or treatment B. They are used in this report because the study is still in progress.

TABLE 2.—CR rate by histologic type

Histology	Treatment					
	X		Y		Totals	
	No. of patients	%CR	No. of patients	%CR	No. of patients	%CR
Embryonal alone or with seminoma	9	67	7	57	16	62
Teratoma alone or with seminoma	5	40	—	—	5	40
Teratocarcinoma with embryonal or choriocarcinoma	4	50	12	42	16	44
Choriocarcinoma with embryonal	5	40	2	0	7	29
Other	—	—	1	0	1	0
Totals	23	52	22	41	45	47

TABLE 3.—CR rate by extent of disease

Extent of disease	Treatment					
	X		Y		Totals	
	No. of patients	%CR	No. of patients	%CR	No. of patients	%CR
Positive markers only	2	50	1	100	3	67
Supraclavicular nodes	1	100	2	100	3	100
Minimal pulmonary disease	4	100	7	71	11	82
Advanced pulmonary disease	10	40	9	11	11	26
Minimal abdominal disease	1	100	0	—	1	100
Advanced abdominal or visceral disease	5	20	3	0	8	12

TABLE 4.—Renal toxicity by treatment group*

	Treatment group	
	X	Y
Highest BUN (mg/100 ml)		
< 20	14	15
21–40	8	2
41–60	0	2
> 60	1	1
Highest creatinine (mg/100 ml)		
≤ 1.2	13	11
1.3–2.0	9	8
2.1–4.0	0	1
> 4.0	1	0

*Data shown are No. of patients in each toxicity category.

TABLE 5.—Hematologic toxicity by treatment group*

	Treatment group	
	X	Y
Lowest wbc count (cells × 10^3/mm^3)		
> 4.5	4	3
3.0–4.5	11	3
2.0–3.0	2	9
1.0–2.0	5	4
< 1.0	1	1
Lowest platelet count (cells × 10^3/mm^3)		
> 130	13	6
90–130	2	9
50–90	6	3
25–50	2	2
< 25	0	0

*Data shown are No. of patients in each toxicity category.

TABLE 6.—Comparison of CR rates in 3 series by histology

Histology	Samuels et al (12)		Einhorn et al (13)		Present study	
	No. of patients	%CR	No. of patients	%CR	No. of patients	%CR
Pure seminoma	11	36	4	75		
Embryonal alone or with seminoma	36	58	25	84	16	62
Teratoma			2	50	5	40
Teratocarcinoma with embryonal or choriocarcinoma	21	33	9	55	16	54
Choriocarcinoma with embryonal	10	20	5	60	7	29
Yolk sac			2	100		

TABLE 7.—Comparison of CR rates in 3 series by anatomic extent

	Samuels et al (12) (No. of CRs/ No. of patients treated)	Einhorn et al (13) (No. of CRs/ No. of patients treated)	Present study (No. of CRs/ No. of patients treated)
Positive markers only		3/3 (100%)	2/3 (67%)
Supraclavicular nodes	4/7 (57%)		3/3 (100%)
Minimal pulmonary disease	9/11 (81%)	9/10 (90%)	9/11 (82%)
Advanced pulmonary disease	12/21 (57%)	6/9 (67%)	5/19 (26%)
Minimal abdominal ± minimal pulmonary disease		8/9 (88%)	1/1 (100%)
Advanced abdominal or visceral disease	3/17 (17%)	9/16 (56%)	1/8 (12%)

for these two groups combined there were only six CRs among 27 patients treated (22% CR). Five patients had brain metastases associated with either advanced pulmonary or abdominal disease and none of these achieved a CR. No significant differences emerged between the two treatment groups.

Toxicity could be evaluated in 23 patients receiving treatment X and in 20 patients receiving treatment Y (tables 4 and 5). Renal toxicity (table 4) was acceptable, with only four patients showing a BUN of > 40 mg/100 ml and only two patients showing a creatinine of > 2.0 mg/100 ml. The most severe reaction included a BUN of 94 mg/100 ml and a creatinine of 7.1 mg/100 ml which was partially reversible. Hematologic toxicity is shown in table 5. No important differences emerge between the two treatment groups. No patient experienced life-threatening thrombocytopenia.

DISCUSSION

The present results are compared with two other series in tables 6 and 7 (12,13). Drawbacks exist when such comparisons are made, including differences in case selection, duration of evaluation, etc. Response is analyzed by histologic type in table 6. In all three series the highest response rate was noted for embryonal carcinoma. Overall, the CR rates for the present series are comparable to those of Samuels et al (12) and slightly lower than those reported by Einhorn et al (13).

A comparison of response by disease extent (table 7) shows high response rates in all series for patients with limited-disease extent. If we combine results for patients with positive markers only, supraclavicular nodes, minimal pulmonary disease, and minimal abdominal disease in the present series the CR rate is 83%. This is comparable to 91% for Einhorn's series and 72% for Samuels' series for a similar combination of patient categories. For advanced pulmonary and abdominal diseases the CR rates in the present series are lower than those of Samuels and Einhorn. This may be due in part to incomplete ascertainment of CR, because for many patients data submission is up to date only for the first two cycles of chemotherapy. Patients in PR after two cycles may achieve CR during continuing chemotherapy, so our CR rate may increase as additional followup is analyzed.

We plan to continue entering patients in this study.

We may wish to consider additional intensive chemotherapy for those patients not achieving CR after two induction cycles as currently given. For example, these patients have received a cumulative dose of bleomycin of 280 mg/m^2 and could receive additional treatment with this agent. Future analyses will include a comparison of durations of remission and survival for the two induction arms of the protocol and for the two continuing treatment arms.

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Sequential Combination Chemotherapy and Surgery for Disseminated Testicular Cancer: *cis*-Dichlorodiammineplatinum(II), Vinblastine, and Bleomycin Remission-Induction Therapy Followed by Cyclophosphamide and Adriamycin [1]

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SUMMARY

A multimodality treatment program for disseminated testicular cancer using vinblastine/bleomycin/*cis*-dichlorodiammineplatinum(II) remission-induction therapy followed by surgery and cyclophosphamide/Adriamycin maintenance therapy has been used in 25 patients. The overall complete and partial response rate was 100%. Six of six patients (100%) with stage II (advanced abdominal) disease have remained in complete remission for a median followup period of 8 months. Seventeen of 19 patients (89%) with stage III metastatic disease have been in complete remission for a median duration of 20 months. No relapses have occurred in any patients who achieved a complete remission. Two of 19 patients (11%) with stage III disease achieved a partial response; one died at 4 months and the other died at 14 months. The toxicity was severe, especially during remission induction. However, there were no drug- or surgically related deaths.

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Although the annual incidence of testicular cancer is approximately 2.1–2.2 cases/100,000 males in the US, and the disease accounts for < 1% of all cancer deaths, it is the most frequent malignancy in men between the ages of 20 and 34 years (1). In 1978, approximately 4000 cases of testicular cancer were diagnosed. Patients with pure seminoma, who account for approximately 40% of these cases, achieve a 90%–95% cure rate when the disease is localized and treated with radical orchiectomy and radiation therapy (2). Until recently, prolonged survivorship and cure rates in patients with nonseminomatous germ cell cancers of the testicle were consistently lower (3).

Chemotherapy and surgery have greatly improved the results of nonseminomatous testicular cancer treatment (4). In 1960, Li et al (5) demonstrated that the combination of actinomycin D, chlorambucil, and methotrexate was effective in achieving 50%–60% response rates, including 15%–20% durable complete responses (CR) in patients with disseminated testicular cancer. Since then, it has been determined that germ cell testicular cancer is responsive, in terms of both overall response and CR rates, to both single agents and combinations of agents of differing structure and mechanism of action. Among the active single agents are vinblastine (6), actinomycin D (7), bleomycin (8), Adriamycin (9), cyclophosphamide (10), and *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) (11). The chemotherapy for testicular tumors was

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further advanced by the demonstration that high doses of vinblastine combined with bleomycin produced responses in approximately 75% of patients, including 40% CR rates, which were not only durable but were also achieved in previously treated patients with advanced disease (12). This observation was derived from the earlier demonstration that high-dose vinblastine (0.4–0.8 mg/kg over 2–3 days [approximately three to six times the usual weekly dose of vinblastine used as a single agent]) was especially active and capable of inducing complete regression of disease (6,12). More recently, *cis*-platinum has been found effective for testicular cancer, with response rates of 66% in previously treated patients. A combination of vinblastine, bleomycin, and *cis*-platinum (VBP) has been studied extensively by Einhorn and Donohue (13) with very encouraging results. In 47 evaluable patients with disseminated disease, an overall response rate of 100%, including a 74% CR rate, was achieved. The addition of surgical removal of residual tumor after induction therapy in five patients increased the CR rate to 85%. Maintenance therapy in patients achieving a CR following induction treatment with VBP included vinblastine and bacillus Calmette-Guérin immunotherapy (Tice-bacillus Calmette-Guérin). However, there have been both drug-related deaths and relapses in this program, with 29 of the original 47 patients (61%) remaining alive without evidence of disease and 34 patients (72%) remaining alive in a 13-month followup.

In 1976, we developed a combination chemotherapy and surgery program in an attempt to prevent late relapses and to increase the ultimate cure rate in patients with disseminated testicular cancer. Based upon the success of the Einhorn and Donohue program (13), we selected a similar VBP induction chemotherapy regimen. We hoped to minimize the problem of acquired resistance to VBP by designing a maintenance chemotherapy program consisting of Adriamycin and cyclophosphamide. Aggressive surgical therapy including modified bilateral thoracoabdominal lymphadenectomy and surgical removal of areas containing bulky disease has also been an integral part of our program. The treatment results in our first 25 patients are the basis of this report.

MATERIALS AND METHODS

Twenty-five patients with disseminated gonadal germ cell tumors were entered in the study. The stage of disease was assessed using the Walter Reed General Hospital (WRGH) staging classification (1). Nineteen patients were stage III and six patients were stage II with massive retroperitoneal lymph node involvement. All 25 patients were evaluated for response and toxicity and patients with extragonadal germ cell disease were excluded.

Treatment Plan

Patients with either advanced stage II or stage III disease received induction chemotherapy with four cycles of VBP over a 12-week period as the initial treatment modality following orchiectomy. After clinical re-evaluation, modified bilateral thoracoabdominal lymphadenectomy and surgical removal of residual disease, if any, were performed in 11 of 25 patients, followed by chemotherapy with cyclophosphamide and Adriamycin. The duration of this second phase of chemotherapy was dictated by the surgical results: patients were treated for 30 weeks (ten cycles) (vide infra) if residual malignancy was found at the time of operation and for 15 weeks (five cycles) if the surgically excised tissue contained only fibrosis, necrosis, or teratomatous transformation (mature teratoma) (14,15).

Five patients with surgical stage II disease had already received a transabdominal retroperitoneal lymphadenectomy prior to referral, while seven patients with surgically proven stage III disease were referred to the Sidney Farber Cancer Institute following thoracotomy, lymphadenectomy, or a scalene lymph node biopsy. Following induction chemotherapy, these patients were thoroughly re-staged with abdominal ultrasound, iv pyelogram, abdominal computerized tomographic scan, whole-lung tomography, tumor markers (α-fetoprotein and β subunit human chorionic gonadotropin), and bone scan. In all 12 patients, these re-staging tests were negative, including the biochemical markers. This group of 12 patients, who had initially received surgery prior to induction chemotherapy, received five cycles of cyclophosphamide and Adriamycin following remission induction; all treatment was then discontinued (table 1).

Induction Chemotherapy

Each cycle consisted of vinblastine (0.2 mg/kg iv bolus) given on Days 1 and 2; bleomycin (30 units iv bolus) given on Days 1, 8, and 15; and *cis*-platinum (20 mg/m^2 iv) given on Days 1–5, accompanied by saline hydration (800–1200 ml of normal saline prior to receiving the drug). Cycles were repeated every 21 days for a total of four cycles, usually spanning a 12-week period. Mannitol diuresis was not used with the *cis*-platinum administration. Bleomycin was discontinued after 360 units.

Operative Approach

The tumor was approached by a thoracoabdominal incision through the ipsilateral chest. If there was no remaining bulky residual tumor, the peritoneum was mobilized as a complete envelope to the contralateral side, exposing both great vessels and the retroperitoneum to the contralateral ureter. If bulky tumor remained, the peritoneum was entered and the posterior

TABLE 1.—Patient status: surgery prior to induction chemotherapy*

Stage	No. of patients with surgery before VBP	Status after surgery		
		No. of CRs	No. of PRs	% CR
II	5	5	0	100
III	7	3	4†	43

*Disease status of 12 patients who received reductive surgery prior to induction chemotherapy with VBP. 4 of 7 patients with stage III disease had pulmonary or supradiaphragmatic lymph node disease remaining after initial surgery but prior to chemotherapy with VBP. One of these 4 partial responders did not achieve CR following VBP chemotherapy and died 4 mos following chemotherapy.
†3 of these patients attained CR following induction chemotherapy.

peritoneum was incised, mobilizing the entire small bowel, ascending colon, and transverse colon on the vascular pedicle of the superior mesenteric artery. The tumor was removed en bloc from the crus of the diaphragm superiorly to the ipsilateral previously ligated spermatic cord inferiorly. The contralateral renal hilum and ureter form the lateral border. The aorta and inferior vena cava were mobilized completely by division of all lumbar vessels, and the lymphatic tissue was removed from the anterior spinous ligament and passed underneath the great vessels (16). This approach allowed complete removal of retroperitoneal lymphatic tissue as well as any residual tumor.

Maintenance Chemotherapy During Remission

Each cycle consisted of cyclophosphamide (500 mg/m^2 iv) and Adriamycin (50 mg/m^2 iv), both given on Day 1. Cycles were repeated every 21 days for a total of either five (15 weeks) or ten (30 weeks) cycles, depending on the re-staging and survival results following induction chemotherapy.

Hematologic, renal (including creatinine clearance), hepatic, and neurologic parameters were routinely monitored during therapy. α-Fetoprotein and the β subunit of human chorionic gonadotropin by radioimmunoassay were obtained, usually during each cycle. Appropriate radiographic, radionuclide, ultrasonic, and computerized tomographic studies were performed before treatment, during every other cycle, and every 4–6 months after completion of treatment.

Response Criteria

A partial response (PR) was defined as a > 50% reduction in the product of the longest perpendicular diameters of all measurable lesions lasting at least 4 weeks. The presence of detectable biochemical markers following either chemotherapy or surgery was considered evidence of active disease; such patients would be considered as having a PR, even if all measurable disease had regressed following chemotherapy or had been resected with surgery. A CR signified that all evidence of disease (as measured by physical examination, radiographs, negative biochemical marker tests, and surgical findings) had completely disappeared for at least 4 weeks. Response duration was measured from Day 1 of induction chemotherapy.

RESULTS

Twenty-five patients were entered in this chemotherapy-surgery program for gonadal germ cell cancer. Six patients were stage II with massive retroperitoneal lymph node disease and were classified as having advanced abdominal disease (without liver metastases), as established by the classification of Samuels et al (17). Nineteen were WRGH stage III at the time of diagnosis. Seven of these patients had had reductive surgery prior to induction chemotherapy with VBP. Twenty-five patients were evaluable for response and toxicity and comprise the present study group.

Stage II Patients

The six patients with WRGH stage II disease have been in CR for a median duration of 8 months (range, 4–17). Five of these six patients were referred to the Sidney Farber Cancer Institute following lymphadenectomy and removal of all gross disease. The remaining patient had a lymphadenectomy after chemotherapy with VBP. Mature teratoma was found and resected, and the patient then received chemotherapy with cyclophosphamide and Adriamycin for 15 weeks (five cycles). All patients in this category have received five cycles of cyclophosphamide and Adriamycin.

Stage III Patients

Nineteen patients with stage III disease have been studied. Seventeen (89%) of these 19 patients remain in CR and without recurrence for a median duration of 20+

TABLE 2.—Patient status: induction chemotherapy prior to surgery

Stage	No. of patients with VBP before surgery	Status after surgery		% CR
		No. of CRs*	No. of PRs	
II	1	1	0	100
III	10	9	1†	90

*All biochemical markers were negative.
†The 1 patient who remained as a partial responder died 14 mos after induction chemotherapy.

TABLE 3.—Pathologic results in stage III patients undergoing lymphadenectomy following VBP therapy

Clinical status after VBP	No. of patients	Pathologic/surgical findings	No. of patients
CR	4	Teratoma/resected	2
		Fibrosis/resected	1
		Teratocarcinoma/resected	1
PR*	6	Teratoma/resected	3
		Fibrosis/resected	1
		Teratocarcinoma/resected	1
		Embryonal carcinoma/unresected	1

*All patients classified as partial responders had negative biochemical markers but had clinical and radiologic evidence of residual bulky disease.

months (range, 3+ to 29+). Two (11%) of the 19 patients had a PR, indicating an overall response rate of 100%. The partial responders died of progressive disease at 4 and 14 months respectively after initiating therapy. Seventeen patients with stage III disease had lymphadenectomy and/or thoracotomy, seven before induction chemotherapy and ten following chemotherapy. Two patients did not undergo lymphadenectomy: one patient presented with disseminated seminoma following abdominal and mediastinal radiation therapy, and one patient had been successfully treated for stage IIIB Hodgkin's disease with MOPP (mechlorethamine, vincristine, prednisone, and procarbazine) chemotherapy and total-nodal radiotherapy 5 years earlier and then presented with disseminated embryonal carcinoma. The status of the ten stage III patients who had induction chemotherapy followed by surgery is shown in table 2.

Three of the 15 complete responders with stage III disease who had surgery and chemotherapy achieved a CR following surgery and prior to induction chemotherapy. Ten patients achieved a CR with the combination of surgery and chemotherapy (three patients received surgery followed by chemotherapy and seven received chemotherapy followed by surgery). Two patients achieved a CR after chemotherapy alone, since the lymphadenectomy following chemotherapy was entirely negative.

The surgical and pathologic findings in the ten stage III patients who had lymphadenectomy following chemotherapy are shown in table 3. It is noteworthy that three of four patients who were thought to be in clinical and biochemical CR following VBP were found to have teratoma or teratocarcinoma at subsequent surgery. Similarly, areas of bulky disease following induction chemotherapy represented either teratoma or fibrosis in four of six patients.

All patients who achieved a CR, either by chemotherapy alone, surgery alone, or the combination of chemotherapy and surgery, are alive with no evidence of either clinical or biochemical marker abnormalities. There have been no relapses among the complete responders.

Response According to Histology and Sites of Disease

Patients with all groupings of the Dixon-Moore germ cell classification responded. The two patients achieving PR were in Dixon-Moore stage II (embryonal carcinoma with or without seminoma) and Dixon-Moore stage V (choriocarcinoma with embryonal carcinoma). Most of our patients were in the Dixon-Moore stage IV (teratoma with embryonal carcinoma) category. The correlation of response with the extent of disease, according to the classification of Samuels et al (17), is shown in table 4. The two patients who achieved only PR came from the advanced abdominal disease category.

TABLE 4.—Response according to extent of disease

Category	No. of patients	% CR
Minimal pulmonary disease	2	100
Advanced pulmonary disease	10	100
Minimal abdominal disease	4	100
Advanced abdominal disease	9	77

Previous Treatment

Four patients with stage III disease had received prior radiation therapy or chemotherapy. All four entered CR. In previously irradiated patients, 75% of the induction vinblastine dose was administered.

Toxicity

Induction Chemotherapy With Vinblastine, Bleomycin, and cis-Platinum

Hematologic toxicity was severe. A wbc count of $< 2000/mm^3$ and a granulocyte count of $< 500/mm^3$ were observed in 61% of the courses. Thrombocytopenia with platelet counts of $< 100,000/mm^3$ was seen in 26% of the courses. Only 3% of the courses were associated with thrombocytopenia alone. The mean nadir granulocyte count was $150/mm^3$ and occurred on Day 10 (median) (range, 7–16); the platelet nadir occurred on Day 7 (range, 2–16). A median hematocrit drop of 14% (range, 5%–20%) occurred in 77% of the patients, many of whom required blood transfusions. Fifty percent of the patients experienced a weight drop of 8 kg (range, 5–19 kg) during induction treatment. Transient nephrotoxicity, manifested by a mean increase in the serum creatinine of 71% above baseline (range, 25%–123%), occurred in 40% of the patients. This was usually reversible within each course. Only saline diuresis was used during the *cis*-platinum administration. No patient had a permanent increase in the baseline serum creatinine of > 25% after completion of induction chemotherapy.

Fever and suspected sepsis were seen in 24% of the patient courses, neuromyalgia was seen in 14%, reversible tinnitus and high-frequency hearing loss were seen in 10%, *Staphylococcus aureus* overgrowth of the mouth was seen in 3%, and jaw pain was seen in 10%. Nausea, vomiting, mild stomatitis, and alopecia were universal findings. Based upon these toxic effects, dose de-escalations were needed in the majority of courses. In 51% of the courses less than full doses of vinblastine were given, and in 15% less than full doses of *cis*-platinum were given. No pulmonary toxicity secondary to bleomycin was observed.

Surgery

Even after aggressive chemotherapy, the operative intervention was remarkably well-tolerated in all patients. The average postoperative hospital stay was 7.2 days, and there were no deaths. In one patient who had had simultaneous wedge resection of a pulmonary nodule, rapid atrial fibrillation required administration of digoxin and quinidine to control his rhythm, with spontaneous conversion to normal sinus rhythm several days later. One other patient had minor atelectasis and a small pleural effusion. There were no episodes of thrombosis or pulmonary embolus.

Maintenance Chemotherapy With Cyclophosphamide and Adriamycin

Cyclophosphamide and Adriamycin were well-tolerated following VBP chemotherapy. Myelosuppression, as defined above, occurred in only 5% of the patient courses. Cardiac toxicity and bladder toxicity were not encountered.

DISCUSSION

A 100% overall response rate with induction VBP and reductive surgery was demonstrated in our study of 25 patients (table 5). No relapses have occurred in our 23 patients who have entered CR. In the initial VBP studies, as described by Einhorn and Donohue (13), relapses usually occurred within 9 months of induction chemotherapy. Our encouraging results are based on a median duration of followup of 8 months in stage II patients and 20 months in stage III patients. Maintenance therapy consisted of the combination of Adriamycin and cyclophosphamide.

The use of surgery has been extremely important in achieving CR in patients following chemotherapy. The fact that ten stage III patients achieved a CR by the combination of intensive chemotherapy and complete surgical removal of residual cancer or teratoma indicates the importance of an aggressive surgical approach. In addition, the remission status in the majority of our patients has been substantiated by both negative biochemical marker studies and surgical findings.

The toxicity of the entire program, especially the induction portion, is substantial. The possibility of diminishing the induction dose of vinblastine seems to be a reasonable alternative, since only 49% of the patients in this series were able to receive full doses of this agent. Surgical intervention following induction chemotherapy has been well-tolerated. In addition, chemotherapy with cyclophosphamide and Adriamycin has been exceptionally well-tolerated and was associated with only minimal myelosuppression.

The optimal approach in the treatment of patients with more than minimal stage II disease (stage B_2) (4) who

TABLE 5.—Testicular cancer: remission induction

Stage	No. of— Patients entered	CRs (%)	Relapses	No. of patients who completed full protocol, then discontinued treatment	No. of PRs
II	6	6 (100)	0	6	0
III	19	17 (89)	0	14	2

have been rendered disease-free following a surgical lymphadenectomy remains to be determined. As noted, we are treating these patients as aggressively as patients with measurable, disseminated disease. In a previously reported series (13), one third of the patients in stage II disease treated with actinomycin D relapsed. The combination of an induction chemotherapy program with VBP and reductive surgery has been successful in preventing any patient in CR with stage II or stage III disease from relapsing.

The surgical findings in patients treated initially with induction chemotherapy indicate that both teratoma or teratomatous transformation and frank malignancy may persist, despite negative biochemical and radiographic evaluations. Although the natural history of teratoma following chemotherapy seems to be favorable, additional followup is needed.

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Chemotherapy for Testicular Cancer: Current Status of the National Cancer Institute Combined Modality Trial

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SUMMARY

Twenty-five previously untreated patients with advanced testicular carcinoma were treated with a five-drug combination chemotherapy program containing *cis*-dichlorodiammineplatinum(II). This drug regimen was used as part of a trial testing the efficacy of cytoreductive surgery (15 patients) and in ten additional patients not eligible for the combined modality study. Eleven of 25 (44%) patients had a complete response and 11 of 25 (44%) patients had a partial response. Nine of 11 (82%) patients with minimal tumor burden upon initiation of chemotherapy had a complete response. With a median followup of 12+ months, none have relapsed. Complete remissions in patients with very advanced bulky disease were rare (two of 14 [14%] patients) and of short duration, with both patients relapsing. In addition to the usual hematologic toxicity, 14 of 25 (56%) patients developed hypomagnesemia and five of 25 (20%) patients developed systemic reactions to *cis*-dichlorodiammineplatinum(II) which prevented further drug administration.

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Chemotherapy for testicular neoplasms represents the first successful combination chemotherapy in man, beginning with the report of Li et al in 1960 (1). Since that initial report in which seven of 23 (30%) patients had significant disease responses and three patients are still alive and free of disease 20+ years later, the efficacy of combination chemotherapy regimens for nonseminomatous testicular cancer has improved dramatically. Results of modern chemotherapy regimens incorporating vinblastine, bleomycin, and *cis*-dichlorodiammineplatinum(II), with or without additional agents, indicate that complete remission rates of 50%–80% can be achieved (2–6). Followup evaluation of patients in these series is relatively short and ultimate relapse rates have not yet been defined. However, it is clear that such chemotherapeutic regimens have made a significant impact upon the survival of testicular carcinoma patients.

While the current chemotherapy regimens are markedly better than previous treatment programs, there are still subgroups of testicular carcinoma patients whose prognosis is worse than that of other patients, ie, patients with choriocarcinoma or embryonal carcinoma with teratomatous elements have lower complete response rates to combination chemotherapy (40%–45%) than patients with embryonal carcinoma with or without seminomatous involvement (66%–84%) (6).

A second set of prognostic variables relates to the initial tumor burden at the time therapy is initiated. Using estimates of number and size of pulmonary metastases and extent and size of retroperitoneal lymph node involvement, patients with a minimal tumor burden have higher complete response (CR) rates to combination chemotherapy (58%–90%) than do patients with a large tumor burden (52%–68%) (6). This latter prognostic variable can be potentially modified and is the basis for the combined modality treatment protocol for nonseminomatous testicular cancer currently conducted by the Medicine and Surgery Branches of the Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute. Based upon the concept that reducing initial tumor burden might increase the subsequent efficacy of combination chemotherapy and might ultimately improve survival (7,8), a prospective randomized trial of chemotherapy with or without preceding cytoreductive surgery is being tested. Preliminary results of this study, to-

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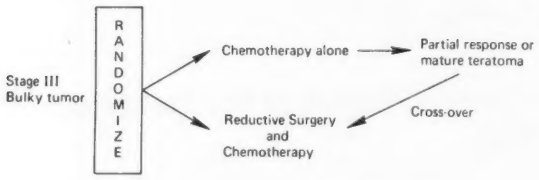

FIGURE 1.—Treatment design for advanced testicular carcinoma.

gether with the chemotherapy results for patients not eligible for this particular trial, will be summarized in this report.

PATIENTS

The design of the National Cancer Institute cytoreductive-chemotherapy protocol is shown in figure 1. To date, 36 patients with advanced (stage II or III) testicular carcinoma have been entered in this study. None had received prior chemotherapy or radiotherapy. Eleven patients still in the induction phase of chemotherapy are excluded from this analysis. Twenty-five patients are currently evaluable for response to therapy. These patients either (*a*) completed the induction portion of the chemotherapy program and are currently receiving the maintenance portion of the treatment schedule; (*b*) relapsed during the induction portion of the program; or (*c*) died subsequent to initiation of therapy. All patients who received surgery and/or chemotherapy were considered evaluable; no patients were excluded because of inadequate duration of therapy. Of the 25 patients, eight were randomized to receive cytoreductive surgery plus chemotherapy and seven were randomized to receive chemotherapy alone. An additional ten patients received chemotherapy alone, including two patients too ill to undergo extensive staging procedures and/or general anesthesia, two patients who had no primary testicular mass and in whom retroperitoneal lymphadenectomy was both diagnostic and therapeutic, three patients lacking bulky disease (thus not eligible for formal randomization) who had surgery for curative intent but showed residual disease after surgery, one patient who had metastatic disease not amenable to debulking therapy (multiple bilateral pulmonary metastases without demonstrable retroperitoneal lymph node involvement), one patient who refused retroperitoneal lymph node dissection, and one patient who had pure choriocarcinoma and advanced pulmonary disease.

The patient characteristics are shown in table 1. The median age of the patients was 27 years, ranging from 15 to 46 years, at the time of referral to the National Cancer Institute. Twenty-three of 25 (92%) patients had stage III disease; 23 of 25 (92%) patients had massive bulky disease as defined by Samuels et al (9), with frequent caval and/or ureteral obstruction, pulmonary insufficiency, gastric outlet obstruction, hepatic metastases, etc. Histologic subtypes are described according to the classification system of Mostofi and Sorbin (10). Of the 23 patients with bulky disease, 13 had a retroperitoneal lymph node dissection, eight on the basis of protocol randomization and five for other reasons as noted above. Chemotherapy was initiated 10–24 days after surgery in these 13 patients.

TABLE 1.—Patient characteristics

	No. of patients
Median age, 27 yrs (range, 15–46)	
Stage (initial tumor burden)	
II	2 (1 bulky, 1 non-bulky)
III	23 (22 bulky, 1 non-bulky)
Histology	
Embryonal carcinoma	5 (20%)
Choriocarcinoma	1 (4%)
Embryonal carcinoma + seminoma	5 (20%)
Embryonal carcinoma + teratoma	9 (36%)
Choriocarcinoma + other elements	5 (20%)

The chemotherapy regimens utilized in these patients are shown in table 2. Chemotherapy regimen I was used in the first 18 patients treated from July 1976 to June 1977. This program was a modification of the VAB III protocol developed at the Memorial Sloan-Kettering Cancer Center (New York, NY), with the induction period including cycles 1–8. After treatment of 18 patients, the regimen was modified based upon the observation that several patients responded to cycles 1 and 2, but relapsed before the second intensive cycle (No. 8). In regimen II, the duration of bleomycin infusion was decreased to 5 days, the doses of cyclophosphamide and *cis*-dichlorodiammineplatinum(II) (*cis*-platinum) were increased from 650 to 1000 and from 70 to 100 mg/m^2 respectively in the intensive cycles, and the number of intensive cycles was increased from two to three (rescheduled to be given as cycles 1, 3, and 5). Bleomycin and low-dose *cis*-platinum were added to the treatment plan on cycles 2 and 4. Maintenance cycles were extended to a total of 24, and included *cis*-platinum administered on even-numbered cycles. No further bleomycin was routinely administered after the fifth cycle. The dose-modification scheme is noted in table 3.

All doses of *cis*-platinum were administered as 30–60-minute infusions following iv hydration (1–2 liters of 5% dextrose in 0.45 normal saline) and mannitol (15 g) diuresis. In addition, patients receiving 70–100 mg/m^2 of *cis*-platinum also received 10–20 mg of furosemide iv im-

TABLE 2.—Chemotherapy regimens
(administered on 21-day cycles × 24 cycles)

Drugs	Regimen
Regimen I (given from July 1976 to June 1977, 18 patients)	
Intensive cycles (Nos. 1 and 8)	
Actinomycin D	1 mg/m², Day 1
Vinblastine	4 mg/m², Day 1
Cyclophosphamide	650 mg/m², Day 1
Bleomycin	15 units/m², Days 1–7 (constant infusion)
cis-Platinum	70 mg/m², Day 8
Nonintensive cycles (Nos. 2–7 and 9–24)	
Actinomycin D	1 mg/m², Day 1
Vinblastine	4 mg/m², Day 1
Cyclophosphamide	100 mg/m², Days 1–14
cis-Platinum	25 mg/m², Day 1 (alternate cycles)
Regimen II (given since June 1977, 7 patients)	
Intensive cycles (Nos. 1, 3, and 5)	
Actinomycin D	1 mg/m², Day 1
Vinblastine	4 mg/m², Day 1
Cyclophosphamide	1000 mg/m², Day 1
Bleomycin	15 units/m², Days 1–5 (constant infusion)
cis-Platinum	100 mg/m², Day 6
Nonintensive cycles (Nos. 2 and 4)	
Actinomycin D	1 mg/m², Day 1
Vinblastine	4 mg/m², Day 1
Cyclophosphamide	100 mg/m², Days 1–10
Bleomycin	15 units/m², Day 1
cis-Platinum	25 mg/m², Day 1
Maintenance cycles (Nos. 6–24)	
Actinomycin D	1 mg/m², Day 1
Vinblastine	4 mg/m², Day 1
Cyclophosphamide	100 mg/m², Days 1–10
cis-Platinum	25 mg/m², Day 1 (alternate cycles)

mediately prior to the *cis*-platinum and an additional 7.5 g of mannitol iv and 1–2 liters of 5% dextrose in 0.45 normal saline after *cis*-platinum. Potassium chloride (15–20 mEq) was routinely added to each liter of 5% dextrose in 0.45 normal saline.

A CR was defined as the complete disappearance of all tumors previously detected by physical examination, iv pyelogram, lymphangiogram, whole-lung tomogram, abdominal sonogram, or computerized tomogram, and a return of human chorionic gonadotropin and alpha-fetoprotein levels to the normal range. Such a response must have lasted ≥ 30 days. A partial response (PR) was defined as a ≥ 50% decrease in all tumors measured as the products of at least two perpendicular diameters.

RESULTS

Of the 25 patients evaluable to date, 11 (44%) achieved a CR, 11 (44%) achieved a PR, and three (12%) had no response to therapy. Although the median followup is of short duration (approximately 1 year), survival differences according to the type of response are already apparent but have not yet reached statistical significance. The median survival (measured from the start of chemotherapy) of patients achieving CR was 15+ months (range, 8+ to 27+) compared to only 8 months (range, 5+ to 20) for patients achieving PR. If this trend is confirmed with additional patient accrual, the difference will undoubtedly increase with further followup since only two complete responders (18%) have relapsed and eight of the 11 partial responders have died. The three patients who were considered nonresponders survived 3, 4, and 16 days respectively (table 4).

Response to therapy is correlated in table 5 with tumor burden at initiation of chemotherapy. Nine of the 11 (82%) patients considered to have minimal tumor burden have had a CR and none of these have relapsed. Of the remaining patients, one had a PR and one died on Day 16 of complications from surgery and chemotherapy. In contrast, only two of the 14 (14%) patients with large tumor burdens achieved a CR; both have relapsed and died. Ten (71%) patients had PRs and two patients died on the third and the fourth day of chemotherapy respectively.

With the exception of the single patient with a pure choriocarcinoma who had only a PR, CRs were achieved in all histologic subgroups, including embryonal carcinoma (20%), embryonal carcinoma plus seminoma (60%), embryonal carcinoma plus teratoma (33%), and choriocarcinoma plus other elements (80%).

The toxicity observed in these 25 patients is shown in table 6. Virtually all patients had alopecia, severe nausea and vomiting, and a ≥ 10-lb weight loss. The latter was only present during the initial induction cycles but nausea and vomiting persisted throughout the entire treatment program. Two patients with CR refused to complete the maintenance portion of their treatment program because of marked nausea and vomiting. Eighty percent of the patients developed at least mild transient mucositis, but in five patients mucositis required prolonged hospitalization for adequate fluid and nutritional support (one patient required parenteral hyperalimentation for 23 days).

Bone marrow impairment was moderate; 15 of 25 (60%) patients had transient leukopenia with wbc counts of $< 3000/mm^3$. However, only four of these patients had a wbc count of $< 2000/mm^3$ and none required hospitalization for infection or febrile episodes associated with periods of leukopenia. Only three (12%) patients had a platelet count of $< 100{,}000/mm^3$ and none required transfusions. Seven (28%) patients developed anemia manifested by a ≥ 3-g/100 ml decrease in hemoglobin.

TABLE 3.—Dose-modification scheme (% of planned dose given)

Toxicity	Cyclophosphamide	Actinomycin D	Vinblastine	Bleomycin	*cis*-Platinum
Hematologic (cells/mm^3)					
Wbc count > 4000	100	100	100	100	100
Wbc count > 3000 but < 4000	50	50	50	100	100
Wbc count > 2000 but < 3000	25	25	25	50	50
Wbc count < 2000	0	0	0	0	0
Platelet count > 100,000	100	100	100	100	100
Platelet count > 50,000 but < 100,000	25	25	25	50	50
Platelet count < 50,000	0	0	0	0	0
Renal (mg/100 ml)					
Creatinine < 1.5	100	100	100	100	100
Creatinine > 1.5 but < 2.0	100	100	100	100	75
Creatinine > 2.0	100	100	100	100	0
Pulmonary					
Pulmonary function tests—					
> 75% of baseline	100	100	100	100	100
> 50% but < 75% of baseline	100	100	100	50	100
< 50% of baseline	100	100	100	0	100

Azotemia was seen in five (20%) patients; it was mild and reversible in three. Fourteen (56%) patients developed hypomagnesemia (serum magnesium levels of < 1.4 mEq/liter) while undergoing induction chemotherapy, including the three patients with mild azotemia and 11 additional patients. Serum magnesium levels as low as 0.6 mEq/liter were noted. All patients initially had normal serum magnesium, with hypomagnesemia developing as early as the second cycle of chemotherapy and as late as the ninth cycle. None of the patients had associated hypocalcemia; none had specific signs or symptoms due to hypomagnesemia.

Three (12%) patients had evidence of hepatic toxicity manifested by transient rises in transaminases; in all three patients this toxicity developed after the first intensive cycle of chemotherapy and did not recur.

Two (8%) patients had fatal multi-organ toxicity. One patient had profound hypoalbuminemia and protein wasting secondary to a thoracic duct fistula after surgery. He developed fever, hypotension, azotemia, and hepatic failure after initiation of chemotherapy and subsequently developed hepatic failure; he died 16 days after therapy was started. Autopsy results demonstrated renal tubular and hepatic necroses. A second patient who relapsed after a PR developed similar complications and pulmonary failure while undergoing harvesting of bone marrow and initiation of ablative chemotherapy with the VAB III-type regimen in anticipation of bone marrow autografting. Autopsy results were the same as those noted in the other patient who died. The exact nature of these complications is unclear. In both patients multi-organ failure developed in the setting of intensive combination chemotherapy including continuous bleomycin infusions, known to produce acute fatal systemic reactions (11). In both patients renal failure developed despite efforts to protect renal function during *cis*-platinum administration with hydration and diuresis.

Five (20%) patients developed systemic reactions to *cis*-platinum. These were manifested by the acute onset (within seconds to a few minutes following the start of

TABLE 4.—Response and survival

Response	No. of responses/ No. of evaluable patients	Survival
No response	3/25 (12%)	3, 4, and 16 days
PR	11/25 (44%)	Median, 8 mos (range, 5+ to 20); 8 of 11 patients dead
CR	11/25 (44%)	Median, 15+ mos (range, 8+ to 27+); 2 of 11 patients dead
Relapse	2/11 (18%)	

TABLE 5.—Response according to tumor volume

Tumor volume	No. of responses (%)
Non-bulky disease (11 patients)	
CR (no relapses)	9 (82)
PR	1 (9)
No response	1 (9)
Bulky disease (14 patients)	
CR (2 relapses)	2 (14)
PR	10 (71)
No response	2 (14)

TABLE 6.—Toxicity (total No. of patients = 25)

Type	No. of patients with toxic effect (%)
Systemic	
Alopecia	25 (100)
Weight loss	24 (96)
cis-Platinum reactions	5 (20)
Gastrointestinal	
Nausea and vomiting	25 (100)
Mucositis	20 (80)
Bone marrow	
Leukopenia (wbcs < 3000/mm^3)	15 (60)
Thrombocytopenia (platelets 75,000–100,000/mm^3)	3 (12)
Anemia (hemoglobin decrease > 3 g)	9 (23)
Renal	
Azotemia (BUN > 25 mg/100 ml, creatinine > 1.5 mg/100 ml)	4 (16)
Hypomagnesemia (magnesium < 1.4 mEq/liter)	14 (56)
Hepatic	4 (16)
Combined renal, hepatic, and pulmonary	2* (8)

*Fatal reactions in both patients.

cis-platinum administration) of anxiety, dyspnea, flushing, and urticarial rashes over the face, thorax, and upper extremities, lasting 30 minutes to several hours. None of the patients developed laryngeal or epiglottic edema, hypotension, or other manifestations of a serious anaphylactic reaction. None were able to tolerate subsequent doses of *cis*-platinum in spite of premedication regimens including oral and iv antihistamines, 72-hour high-dose steroids, or both.

DISCUSSION

The efficacy of combination chemotherapy for testicular cancer has improved dramatically since the initial publication of Li et al (1). With the introduction of vinblastine and bleomycin the CR rate increased from 5%–16% to 22%–36% (6). With newer regimens containing vinblastine and bleomycin (3), and particularly with those new regimens containing *cis*-platinum, the CR rate in large series of patients varies between 50% and 80% (4–6), depending upon the characteristics of the patients entering the protocol. What has become apparent upon analysis of these newer regimens is that patients with favorable histologic subtypes (ie, embryonal carcinoma with or without seminomatous elements) and low tumor burdens have very high CR rates (66%–85%), while patients with unfavorable histologic subtypes (ie, choriocarcinoma with or without other cell types or embryonal carcinoma plus teratomatous elements) and large tumor burdens have lower CR rates (40%–60%) (6).

Since prolonged survival in metastatic testicular cancer is dependent upon achieving a CR, aggressive therapeutic programs are necessary. The efficacy of current regimens is exceptionally good for those patients who are young, have no complicating illnesses, have favorable histologies, and have a minimal tumor burden. Our data are consistent with these observations. Nine of 11 (82%) patients with minimal tumor burdens have achieved a CR. Although seven patients are still completing their maintenance therapy, none have relapsed to date, and their median survival will be > 15 months. Only one patient receiving a complete course of induction chemotherapy failed to achieve a CR (alive with progressive disease at 7 months), while one patient died at 16 days secondary to surgically and chemotherapeutically induced renal and hepatic failure.

In contrast, the survival of patients with advanced bulky disease and/or unfavorable histologic subtypes is significantly worse. Of those patients who had massive tumor burdens (> 50% had obstruction of inferior vena cava and/or at least one ureter; 12 of 14 had advanced pulmonary metastases), only two (14%) achieved CR; both patients have already relapsed. It must be emphasized that these patients represent the extremely advanced cases, eg, gastric outlet obstruction and/or porta hepatis obstruction secondary to extensive retroperitoneal disease, pulmonary insufficiency secondary to multiple metastases (two patients dying 3 and 5 days after the start of chemotherapy), etc.

One approach to treating patients with such unfavorable prognoses would be to increase the intensity of the chemotherapy regimen. As noted in table 6, the toxicity of our particular regimen is significant. Nausea and vomiting are universal and severe; they are now among the dose-limiting toxic effects of *cis*-platinum and several of our patients have refused further chemotherapy. Increasing the dose of *cis*-platinum much more may produce more significant renal toxicity, may potentiate bone marrow toxicity, and may produce an unacceptable level of toxicity which will reduce patient compliance if multiple cycles of therapy are required. Mucositis was observed in 80% of the patients; although it was only mild to moderate in many patients, those patients in the most debilitated state with the most advanced disease had the most severe and prolonged mucositis. Even at these doses total parenteral nutrition was required for one patient. While bone marrow toxicity has been moderate with our regimen, it has been severe in those programs which contain doses of vinblastine of 0.4 mg/kg (3,4). Higher doses of bone marrow-suppressive agents which also produce mucositis may increase the risk of serious bacterial and/or fungal infections. It is likely that such programs will require utilization of protected environ-

ments and/or bone marrow autografts to minimize the risks of infectious complications. To date, the only program beginning to explore such possibilities is being performed by Samuels et al (9) and definitive results are not yet available.

An alternative approach to managing those patients with advanced disease is to utilize the combined modality concept of cytoreductive surgery with chemotherapy. Since the cytotoxicity of chemotherapeutic agents is based upon first-order kinetics, reducing the tumor burden prior to chemotherapy with cytoreductive surgery should improve the efficacy of the chemotherapy. This concept has been proven to be valid in animal models (7,8). Since chemotherapy is highly effective in patients with a small tumor burden, the concept of cytoreductive surgery and chemotherapy deserves a scientific test to determine whether the morbidity and mortality of such a procedure are offset by an increased survival rate following subsequent chemotherapy. The results of this aspect of the National Cancer Institute program are not yet definitive since the number of patients entered in the formal randomized portion of the study is still small and the followup time is still short. Even at this early date, however, the results of the randomized cytoreduction trial appear promising; none of seven patients receiving chemotherapy alone have achieved a CR, compared to five of eight (62%) patients treated with cytoreductive surgery plus chemotherapy. Since none of the five complete remitters have relapsed, and since four of the seven (51%) patients having received chemotherapy alone have already died of recurrent tumor and/or toxicity, it is anticipated that statistically significant differences in CR rates and survival will be achieved within the next 12 months.

Another aspect of this study which is of interest is the high rate of systemic reactions to *cis*-platinum (20%); this is somewhat higher than those rates reported in other series (12) or noted in our institution with protocols utilizing *cis*-platinum either alone or in combination with other cytotoxic agents. Also of interest is the inability to give further doses of *cis*-platinum after such reactions occur; this also is in contrast to the experience of others using *cis*-platinum alone or in other combinations.[2]

In summary, intensive chemotherapy for patients with minimal tumor burdens of testicular cancer is highly effective (82% CR rate and no relapses). In contrast, patients with large tumor burdens clearly have a worse prognosis (14% CR rate with relapses documented). Further followup is expected to verify the efficacy of the cytoreductive-chemotherapy approaches and may elucidate the nature of the renal tubular toxicity manifested by hypomagnesemia. Investigations into the mechanism(s) of *cis*-platinum systemic reactions must be undertaken.

[2]Corder M. Personal communication.

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Basis for Adjuvant Chemotherapy for Stage II Testicular Cancer

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For the Intergroup Testicular Protocol Writing Committee

The problem of adjuvant chemotherapy for testicular cancer can be seen in numeric perspective by reference to the estimates for a hypothetic series shown in table 1. While series will differ in the frequency distribution and cure rates reported, the conclusion remains that a significant percent of patients eventually diagnosed with stage III disease were previously in stage I or II and thus would have been possible candidates for adjuvant chemotherapy.

The requirements for a successful adjuvant chemotherapy program include the availability of effective chemotherapy based on results in advanced disease and the ability to identify patients who are at high risk for development of metastases. Numerous published reports and the papers presented in this issue attest to the availability of effective chemotherapy for testicular cancer. Results include a high frequency of complete response for stage III disease and a projection that more than one half of the patients achieving complete response will be long-term survivors. The patients at high risk for development of metastases are predominantly those with stage II disease at presentation. Also, a smaller fraction of patients with stage I disease will develop metastases, and one of the secondary objectives of the proposed adjuvant protocol will be an attempt to identify prognostic variables which would predict those stage I patients who are at risk for metastases.

In reviewing the results of chemotherapy for testicular cancer it is apparent that a major determinant of complete response is the extent of disease, with the highest complete response rates noted in patients with limited pulmonary disease and in patients with only elevated human chorionic gonadotropin. Extrapolation from this correlation suggests that the highest chance of cure exists when treating microscopic metastases. This hypothesis is supported by the results of Skinner (1) and those of Vugrin et al at the Memorial Sloan-Kettering Cancer Center (2). Skinner has reported an overall survival rate of 90% for patients with stage I and II testicular tumors using retroperitoneal lymphadenectomy and chemotherapy. In the Vugrin series (2), 25 patients who had five or fewer lymph nodes involved with no node > 2 cm in diameter have all remained free of disease recurrence at a median of 10 months after moderate-intensity chemotherapy. However, 11 of 35 patients with less favorable stage II disease (more than five nodes with nodes > 2 cm in diameter) have relapsed after moderate-intensity chemotherapy with 17 months' followup. A group of 11 patients also having less favorable stage II disease (more than five nodes or nodes > 2 cm in diameter) were treated with intensive chemotherapy, with only one relapse with a median of 6 months' followup (2). These observations support the need for full-dose, intensive chemotherapy in adjuvant therapy.

TABLE 1.—Estimates of stage distributions for testicular cancer

Disease stage	Estimated stage distribution of patients (%)		No. of patients eventually stage III
	Initial presentation	Cure with surgery ± radiation	
I	45	90	5
II	35	60	14
III	25		25

TABLE 2.—Accuracy of lymphangiograms in testicular cancer*

Lymphangiogram	Histologic results	
	Positive	Negative
Positive	101	21
Negative	46	128

False-positive rate = 21/101 + 21 (17%)
False-negative rate = 46/46 + 128 (26%)

83% of lymphangiogram-positive patients had positive histology

26% of lymphangiogram-negative patients had positive histology

*Data pooled from 8 series (refs 3–9).

[1]Medical Oncology Section, Department of Medicine, and the Cancer Center, Northwestern University School of Medicine, Chicago, Ill.

[2]I wish to thank Carole Enge for her excellent secretarial assistance.

**Reprint requests to:* Dr. William D. DeWys, National Cancer Institute, Landow Bldg, Rm 8C17, National Institutes of Health, Bethesda, Md 20205.

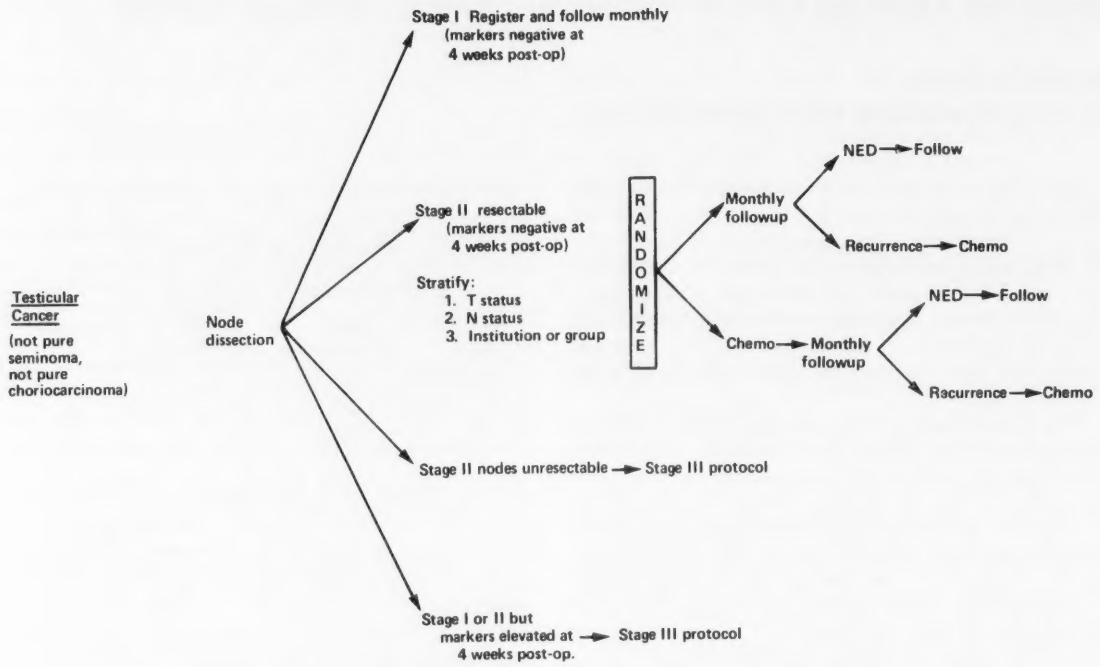

FIGURE 1.—Schema for the proposed intergroup stage I and II testicular protocol.

However, the use of toxic chemotherapy must be weighed against the consideration that a significant percent of stage II patients may be cured with orchiectomy and retroperitoneal node dissection, and that patients who do have disease recurrence may have a chemotherapeutic cure even with recurrent or metastatic disease. Because of this potential for cure of metastases, we cannot, a priori, conclude that adjuvant chemotherapy will be better than chemotherapy administered selectively only to those patients who develop recurrence. Data supporting this approach are those of Einhorn who has followed 52 stage II patients diagnosed between 1973 and 1976 (Einhorn L, personal communication, 1978). Eighteen of these 52 patients developed metastases, and all 18 achieved complete remission with VBP (vinblastine, bleomycin, and *cis*-platinum) chemotherapy. Sixteen patients remain free of disease, one died of progressive tumor, and one died of *Klebsiella* pneumonia 4 months after chemotherapy. An important aspect of Einhorn's protocol was the monthly followup examination including chest roentgenogram and serum marker determination.

Based on the above data, an intergroup protocol development committee has prepared a protocol, the schema of which is shown in figure 1. Patients with stage I disease will be registered and closely followed with the goal of identifying risk factors which may predict which patients will develop metastases. Patients with stage II disease, not pure seminoma and not pure choriocarcinoma, and with resectable retroperitoneal nodes with normal serum markers at 4 weeks after lymphadenectomy will be randomly assigned to chemotherapy or monthly followup with chemotherapy for disease recurrences. Patients with unresectable stage II disease or with persistently elevated markers were included in an early draft of the protocol but are now excluded because of the more complex therapy required for these patients.

The chemotherapy will be either modified VAB IV (vinblastine, actinomycin D, bleomycin, cyclophosphamide, and *cis*-platinum) or VBP, with each group or institution choosing one of these two regimens. The selection of patients is based on surgical staging rather than lymphangiographic staging, based on the inherent error rate of lymphangiography as summarized in table 2 which shows pooled data from eight series of lymphangiography in testicular carcinoma (3–9). Note the false-positive rate of 17% and the false-negative rate of 26%. We will also encourage use of ultrasonographic and computerized tomographic scanning before lymphadenectomy. Improved staging with these modalities may make a future protocol not requiring lymphadenectomy feasible. This would permit comparison of lymphadenectomy versus radiotherapy or evaluation chemotherapy without lymph-

adenectomy in future protocols.

The stage II adjuvant chemotherapy protocol was activated in May 1979. We encourage the full participation of all members from all cooperating groups. (The full text of the chemotherapy protocol may be obtained by writing to the author.)

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ANNOUNCEMENTS

EORTC Conference on Metastasis: Clinical and Experimental

London, England, April 21–23, 1980

The European Organization for Research on Treatment of Cancer (EORTC) Metastasis Group will present a conference on Metastasis: Clinical and Experimental to be held at the Royal Institution, Albermarle St, London, W1, England, on April 21–23, 1980. There will be invited papers and free communications on metastatic potential, experimental models, host factors, therapy, blood coagulability, and clinicopathologic aspects.

For further information contact: **Secretariat, K. Hellmann, DM, Cancer Chemotherapy Department, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2, England.**

2nd Workshop on Cloning Human Tumor Stem Cells

Tucson, Arizona, January 3–5, 1980

A workshop on soft agar cloning methods for human tumor stem cells will be held at the University of Arizona College of Medicine in Tucson, January 3–5, 1980, under the sponsorship of the university's Cancer Center Division. The workshop chairman will be Dr. Sydney E. Salmon, Director of the Cancer Center. In addition to local faculty, guest faculty will include Drs. Anne Hamburger, Daniel Von Hoff, and Ronald Buick.

Further information may be obtained by writing to: **Cancer Center Division, University of Arizona, Health Sciences Center, Tucson, AZ 85724, or by phoning the Conference Coordinator, Mrs. Mary Humphrey, at (602)626-6044.**

International Symposium on New Trends in Human Immunology and Cancer Immunotherapy

Montpellier, France, January 17–19, 1980

An International Symposium will be held in Montpellier, France, on January 17–19, 1980. The main fields include: phenotype and functions of human lymphocyte subpopulations; characterization and role of the Fc receptor (IBF, K, and NK cells and immune complexes); human lymphoblastoid cell lines as models of differentiation; immune status of cancer patients; and immunotherapy for human cancer (interferon, thymosin, transfer factor, and levamisole). This symposium is organized under the auspices of DGRST by Dr. B. Serrou (Montpellier, France) and Dr. C. Rosenfeld (Villejuif, France). Papers presented will be by invitation only. Due to the limited number of places, only the first 150 participants to register will be accepted. The registration fee is 800 French francs. ***For payment of registration or for more information write to:*** **Dr. B. Serrou, Centre Paul Lamarque, 2, Avenue de Bertin Sans (Cliniques St-Eloi) Boîte Postale 5054, 34 033, Montpellier, Cédex, France.**

Postgraduate Course on Metabolic and Nutritional Care

San Francisco, California, December 9–11, 1979

The Department of Surgery of the University of California School of Medicine, San Francisco, is presenting a postgraduate course on Metabolic and Nutritional Care sponsored by Extended Programs in Medical Education to be held at the

Fairmont Hotel in San Francisco, California, December 9–11, 1979. This course is designed to provide a comprehensive orientation to nutritional assessment and care of patients. The program provides in-depth basic science background for proper utilization of complex modalities of nutritional support. Although the course is primarily designed for the graduate education of physicians of all medical specialties, nurses, dietitians, and pharmacists will be provided with theoretic and practical background applicable to their specialties as well. It is hoped that hospitals involved in the complex methods of nutritional support will elect to send representatives. The curriculum has been designed to focus on a wide range of subjects including basic nutritional biochemistry, a practical approach to problems of malnutrition and obesity, as well as practical aspects of establishing nutritional support services. Particular emphasis is placed on the role of hyperalimentation and a modular approach to metabolic care of hospitalized patients. The clinical relevance of new nutritional modalities as well as the application of new research in metabolism, cancer, immunology, and multiple organ system failure will be discussed in the context of nutritional therapy. Questions will be answered on panels and controversial areas will be critiqued by experts in the field as presentations are made. Additional panels will be provided to allow experts to participate in identifying common problems in patients which include the management of complications. The final day of the course will focus on the role of nutritional intervention in specific disease states. Printed material will include abstracts of lectures, patient problems, and a metabolic approach to patients with critical illnesses.

This continuing medical education offering meets the criteria for 18 credit hours in Category I of the Physician's Recognition Award of the American Medical Association and the Certification Program of the California Medical Association and is also acceptable for 18 elective hours by the American Academy of Family Physicians. Application has been made for credit from the American Dietetic Association and the California State Board of Pharmacy. This program has also been approved by Continuing Education in Nursing of the University of California School of Nursing, San Francisco, for 1½ units (18 hours) University Extension Credit (X 404.7) or 18 contact hours, BRN Provider No. 00181.

The registration fee is $225.00 for regular registrants, $112.00 for non-University of California at San Francisco interns, residents, and fellows (with letter of verification), and $125.00 for nurses. Advance registration is advised. Hotel accommodations are available on a first-come first-served basis. ***For an application for enrollment and program information contact:*** Extended Programs in Medical Education, University of California, 1308 3rd Ave, San Francisco, CA 94143 (or phone 415-666-2894, 666-2483 [for registration information], or 666-4251 [for program information]).

1978 Manual for Staging of Cancer

The American Joint Committee for Cancer Staging and End Results Reporting (AJC) has published a manual which contains all of the staging systems which have been approved by the AJC plus introductory chapters on the philosophy of the staging of cancer and the proper calculation of survival rates. Copies of the manual may be obtained by writing to the AJC at 55 East Erie St, Chicago, IL 60611.

American Board of Internal Medicine Examinations

The next examinations in Hematology, Infectious Disease, Nephrology, Pulmonary Disease, and Rheumatology will be on June 17, 1980. Registration for these subspecialty examinations will begin August 1, 1979, and will continue through November 1, 1979. For those who began their residency training in internal medicine before June 1, 1970, and who have < 2 years of acceptable subspecialty training this will be the last opportunity for admission to a subspecialty examination. ***Applications may be obtained from:*** The American Board of Internal Medicine, University City Science Center, 3624 Market St, Philadelphia, PA 19104 (telephone: 215-243-1500).

The American Board of Internal Medicine has announced a significant change in policy regarding its training requirements. This change will affect physicians desiring admission to a subspecialty examination who began their residency training in *internal medicine* before June 1, 1970. At present, these physicians are considered to have met the training requirements for admission to a subspecialty examination after completing 1 year of acceptable training in the subspecialty. If once informed of admission to a subspecialty examination, these physicians *will continue* to be considered to have fulfilled the Board's training requirements in that subspecialty. Beginning with the subspecialty examination offered in 1981, however, *all physicians* not previously admitted to an examination will be required to meet the same training requirements which apply to physicians beginning their residency training after June 1, 1970. These requirements currently involve 2 full years of acceptable training in the subspecialty. A complete description of the Board's policies and procedures may be obtained by directing a written request to the American Board of Internal Medicine (above address).

European Symposium on Lung Cancer

Chalkidiki, Greece, September 7–13, 1980

A European Symposium on Lung Cancer to be held September 7–13, 1980, in Chalkidiki, Greece, is being organized by the Greek Radiological Society under the auspices of the European Association of Radiology. *For further information contact:* Secretariat, Department of Radiology, Areteion Hospital, 76 Vass. Sofias, Athens, Greece.

Symposium on the Nutrition of the Cancer Patient

Bethesda, Maryland, January 10–11, 1980

A Symposium on the Nutrition of the Cancer Patient, sponsored by the National Cancer Institute, will be held at the National Institutes of Health, Building 10, Masur Auditorium, Bethesda, Maryland, January 10–11, 1980. Basic and clinical presentations will be given relating to anorexia, host/tumor metabolism, and nutritional support of the cancer patient. *For more information and registration contact:* Jean Cook, Capital Systems Group, 6110 Executive Blvd, Rockville, MD 20852 [telephone, (301)881-9400].

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